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Scientific Opinion on the risks to animal and public health and the environment related
to the presence of nickel in feed**

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SCIENTIFIC OPINION

Scientific Opinion on the risks to animal and public health and the environment related to the presence of nickel in feed¹

EFSA Panel on Contaminants in the Food Chain (CONTAM)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the risks to animal and human health and the environment related to the presence of nickel (Ni) in feed were assessed by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel). The presence of Ni in feed can arise from both natural and anthropogenic sources. Additionally, certain feed materials contain metallic Ni, since it is used as a catalyst in their production. Based on the differences observed between the Ni exposure levels estimated for different animal species and identified no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs), the CONTAM Panel concluded that any adverse impact of Ni via feed to cattle, pigs, rabbits, ducks, fish, dogs, chickens, horses, sheep, goats and cats is unlikely. Concerning the assessment of human health risks from the presence of Ni in food of animal origin, the CONTAM Panel concluded that in the average population the current levels of chronic exposure to Ni, considering only foods of animal origin, might be of potential concern in the young population, in particular in ‘Toddlers’. In the highly exposed population (95th percentile), the concern also extends to the age class ‘Other children’. Regarding acute dietary exposure, the CONTAM Panel concluded that Ni-sensitized individuals are also at risk of developing eczematous flare-up skin reactions through the consumption of food of animal origin. The contribution of food of animal origin to human dietary exposure to Ni should therefore not be underestimated, particularly in age classes with high dietary exposure to Ni. Release to the environment from manure, resulting from its presence in animal feed, is not a major contributor of Ni deposited onto agricultural soils or to the environment.

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KEY WORDS

nickel, feed, human health risk assessment, animal health risk assessment

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SUMMARY

Following a request from the European Commission, the risks to animal and human health and the environment related to the presence of nickel (Ni) in feed were assessed by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel).

Ni is a metal that occurs in a number of different soluble and particulate forms, which are ubiquitously found in the environment both from natural occurrence and from anthropogenic activity. Animal feed contains Ni, particularly in the divalent form, its most stable oxidation state. Additionally certain vegetable oils can contain traces of metallic Ni, since it is used as a catalyst in their hydrogenation.

Following a call for data, two European countries, submitted 1 813 results on Ni concentrations in feed of which 1 794 were from one country. All results were reported as total Ni, with no differentiation between Ni species. Feed related exposure to Ni was estimated by applying two different scenarios, namely (i) exposures based on Ni concentrations in compound feed and forages and (ii) exposures derived from Ni in feed materials including hydrogenated vegetable oils. For compound feed, 317 results were provided, though the livestock species for which these feeds had been manufactured were not given. Assuming that the values reported were representative across species, the same values for compound feed were used for all livestock species. Based on the reported mean concentrations in compound feed and forage, the estimated mean upper bound exposures ranged from 5.1 (fattening beef cattle) to 61.7 µg/kg body weight (b.w.) per day (for laying hens and chickens for fattening). In an alternative scenario, a 5 % inclusion of hydrogenated vegetable oil in the non-forage feeds, containing the maximum acceptable concentration of 50 mg/kg Ni, was assumed and applied to rations for different livestock species used to assess exposure. This, together with levels of Ni in individual feeds provided worst case exposure assessments resulting in mean upper bound exposures to Ni of 0.06 mg/kg b.w. per day for cattle, 0.18 mg/kg b.w. per day for pigs and ducks, 0.01 mg/kg b.w. per day for fish, 0.04 mg/kg b.w. per day for dogs, 0.20 mg/kg b.w. per day for chickens, 0.08 mg/kg b.w. per day for sheep, 0.16 mg/kg b.w. per day for goats, 0.04 mg/kg b.w. per day for horses, 0.11 mg/kg b.w. per day for turkeys and 0.04 mg/kg b.w. per day for cats.

The CONTAM Panel acknowledged that in practice, exposure from hydrogenated vegetable oils is likely to be substantially lower, since according to industry data the median level of Ni in hydrogenated vegetable oils is much lower than the permitted maximum content of 50 mg/kg that was used in the abovementioned worst case exposure assessment. However, these data were not available within the timeframe of this mandate and therefore were not used in animal exposure estimates.

The contribution of water to the overall exposure of livestock was estimated using Ni concentrations in tap water as reported in the European Food Safety Authority (EFSA) data base. Contribution of water was very low.

The CONTAM Panel concluded that under certain conditions, soil ingestion could contribute considerably to Ni intake in the case of foraging animals but since the extent to which this occurs is unclear, soil was not considered in the exposure assessment.

Animals absorb only a small proportion of the total oral Ni intake. After absorption, Ni is rapidly distributed to different organs and can cross the placental barrier. Ni is mainly excreted via the urine and may be excreted also via milk.

Only a limited number of Ni toxicity studies are available for livestock and fish where Ni induced mainly (i) reduced feed consumption and body weight (growth); (ii) reduced relative organ weights (liver and kidney); and (iii) histopathological changes in liver and kidney and/or altered blood parameters. In poultry, in addition to other adverse effects, reproductive toxicity was elicited by Ni. In dogs, marked polyuria, lung lesions and granulocytic hyperplasia of the bone marrow was observed. For cattle a no observed adverse effect level (NOAEL) of 1.34 mg/kg b.w. per day was identified based on findings of reduced feed intake and growth. For pigs a NOAEL of 12.8 mg/kg b.w. was

identified based on reduced feed intake and body weight gain. For rabbits a NOAEL of 3.75 mg/kg b.w. per day was identified based on reduced relative weights of liver, kidneys, ovaries, reduced ovary function and altered blood parameters in female animals. For ducks, a NOAEL of 9.4 mg/kg b.w. per day was identified based on decreased bone density. For fish a NOAEL of 0.2 mg Ni/kg b.w. per day was derived based on histopathological alterations in the kidney. For dogs a NOAEL of 18.0 mg Ni/kg b.w. per day was identified based on vomiting, polyuria, lung lesions and bone marrow hyperplasia. For chickens a reliable NOAEL could not be derived but a lowest observed adverse effect level (LOAEL) of 3.0 mg Ni/kg b.w. per day was identified based on slightly reduced growth, slightly reduced relative weights of livers and testicles and mild pathological liver focal fatty infiltration together with a decrease of specific blood parameters.

As no toxicity studies were identified for turkeys, sheep, goats, horses, and cats no NOAELs/LOAELs could be derived for these species.

The NOAELs/LOAELs derived from the available toxicity studies are much higher than the estimated chronic exposures to Ni. Taking into account the conservative approach adopted in the present opinion for estimating exposures, the CONTAM Panel concluded that any adverse impact of exposure to Ni in feed of cattle, pigs, rabbits, ducks, fish, chicken and dogs is unlikely.

Although for turkeys no NOAEL/LOAEL is available the CONTAM Panel concluded, based on the considerable margin between worst case exposure levels and the NOAELs and LOAELs derived in other poultry species (i.e. chickens and ducks), that any adverse impact on turkeys by Ni in feed is unlikely. No NOAELs/LOAELs could be derived for goats, sheep and horses, but since exposure levels for these species are considerably lower than the NOAEL for Ni in cattle the CONTAM Panel concluded that any adverse effects of Ni in feed are unlikely in these species. Similarly, no NOAEL/LOAEL could be identified for cats but since the exposure level derived for this species is considerably lower than the NOAEL derived for dogs, the CONTAM Panel concluded that any adverse effects from Ni in feed are unlikely to occur.

For the assessment of human health risks from the presence of Ni in food of animal origin, the appropriate occurrence data were extracted from the CONTAM opinion on Ni in food published in 2015. Both chronic and acute dietary exposures estimated in the current opinion were compared with the health based guidance value/reference point derived in the CONTAM opinion on Ni in food published in 2015.

The highest chronic dietary exposure to Ni considering specifically food of animal origin was estimated in 'Toddlers', with values that ranged between 0.9–3.8 µg/kg b.w. per day (lower bound (LB)–upper bound (UB)) for mean dietary exposure and 1.6–5.5 µg/kg b.w. per day (LB–UB) in the highly exposed population (95th percentile). The CONTAM Panel concluded that in the average population the current levels of chronic exposure to Ni considering only foods of animal origin might be of potential concern in the young population, particularly in 'Toddlers'. When assessing the highly exposed population (95th percentile), the CONTAM Panel concluded that the exposure to Ni from foods of animal origin might be of potential concern not only in 'Toddlers', but also in the age class 'Other children'.

Without considering infants, for which only two dietary surveys were available, the average contribution of the foods of animal origin to the mean chronic dietary exposure to Ni (at the LB estimations) ranged between 9.4 % (lowest LB in 'Other children') and 29.1 % (highest LB in 'Toddlers'). 'Milk and dairy products' was one of the main contributors to the chronic dietary exposure to Ni in the young population, particularly in 'Toddlers'.

In 'Adults', high consumption of three representative foods (liquid milk, livestock meat and fish) led to acute dietary exposure estimations of 0.4 µg/kg b.w. per day, 0.9 µg/kg b.w. per day and 0.6 µg/kg b.w. per day, respectively. In 'Toddlers', high consumption of liquid milk led to acute dietary exposure estimates of 1.9 µg/kg b.w. per day. Based on these single-point estimates, the CONTAM

Panel concluded that Ni-sensitized individuals may be at risk of developing eczematous flare-up skin adverse hypersensitivity reactions as a result of the consumption of food of animal origin.

The CONTAM Panel concluded that, while a comprehensive assessment of the environmental impact of Ni in livestock manures is outside the scope of this opinion, the available data suggests that Ni in feed, and subsequently excreted in manure, is not a major contributor of Ni onto agricultural soils or the environment.

Since a carry-over could not clearly be determined due to lack of appropriate studies, the CONTAM Panel concluded that such studies are needed to enable determination of carry-over of Ni from feed to food products of animal origin.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Fatty acids esterified with glycerol and mono-, di- and triglycerides of fatty acids are feed materials listed in the EU Catalogue of feed materials.⁴ Mono-, di- and triglycerides of fatty acids is a product consisting of mixtures of mono-, di- and tri-esters of glycerol with fatty acids, which may contain small amounts of free fatty acids and glycerol. Both feed materials may contain nickel from the hydrogenation process in which nickel is used as a catalyst. In the EU Catalogue it is foreseen that the two feed materials may contain up to 50 mg/kg nickel from hydrogenation with the requirement to declare the nickel content where the content is higher than 20 mg/kg.

When this provision was discussed at the Standing Committee on the Food Chain and Animal Health, section Animal Nutrition concern was expressed for animal and public health as regards the presence of nickel in feed (feed materials and compound feed⁵). Therefore, the Commission representative committed at the meeting to refer a request to EFSA to assess the risks for animal health, public health and the environment of the presence of nickel in feed (feed materials and compound feed), in view of possible regulatory measures on the presence of nickel in feed in the frame of Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in feed.⁶

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002 the Commission asks EFSA for a scientific opinion on the risks to animal and human health and the environment related to the presence of nickel in feed.

The scientific opinion should, *inter alia*, comprise the:

- a) evaluation of the toxicity of nickel for the different animal species of relevance.
- b) estimation of the exposure of the different animal species to nickel from feed.
- c) assessment of the animal health risks for the different animal species as the consequence of the estimated exposure of nickel from feed.
- d) evaluation of the carry-over of nickel from feed to the food products of animal origin.
- e) assessment of human health risks of the presence of nickel in food of animal origin in relation to the exposure to nickel from food other than from animal origin and from other sources.
- f) insofar relevant, assessment of the risk for the environment in relation to the presence of nickel in feed.

⁴ Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1–64.

⁵ A mixture of products of vegetable or animal origin in their natural state, fresh or preserved, or products derived from the industrial processing thereof, or organic or inorganic substances, whether or not containing additives, for oral feeding in the form of a complete feed.

⁶ OJ L 140, 30.5.2002, p. 10.

ASSESSMENT

1. Introduction

Nickel (Ni) is a metallic element which can exist in oxidation forms -1, 0, +1, +2, +3 and +4. In biological systems Ni^{2+} predominates. The forms of Ni occurring in foods and feeds have often not been determined.

Ni is generally considered not to be an essential nutrient in animals. Although several studies have been carried out, usually with low levels of Ni as a dietary supplement, to investigate essentiality of Ni in several livestock species (Spears, 1984; Bersényi et al., 2004; Prasad and Gowda, 2005; Schaumlöffel, 2012), the present opinion does not address these issues and only deals with the risks for animal and public health and the environment related to the presence of Ni in feed.

Extracted vegetable oils are frequently added to livestock diets, principally to increase the energy content of the ration but also as a manufacturing aid in the case of compound feeds. The oils may be hydrogenated in order to improve their handling characteristics and stability, but the process involves the use of Ni catalysts, and as a result, trace amounts of Ni have been reported in hydrogenated vegetable oils. Where hydrogenated vegetable oils are used in livestock diets, the Ni they contain adds to the exposure from that naturally present in feed materials.

1.1. Interpretation of the Terms of Reference

The background to the terms of reference refers to the possible presence of Ni in feed materials defined in the EU Catalogue of feed materials as a result of processing methods involving use of metallic Ni as a catalyst. The EU Catalogue of feed materials⁷ lists in total seven feeds derived from fatty acids, for which elevated levels of Ni might be expected as a result of this processing method.

While considering the exposure to Ni from these feeds in particular it should be noted that the present opinion constitutes a risk assessment for animal health related to the presence of Ni in all feed materials.

The present document comprises an evaluation of Ni toxicity in animal species of relevance, together with an assessment of exposure to Ni from feed and the resulting health risk assessment for these species. It also contains an evaluation of the carry-over of Ni from feed to food of animal origin and an assessment of the contribution of Ni from feed to the environment. Following the terms of reference, an assessment of exposure and resulting human health risks related to the presence of Ni in animal derived food has been carried out that is based on the scientific opinion on the risks to public health related to the presence of Ni in food and drinking water (EFSA CONTAM Panel, 2015), which is hereinafter referred to as the opinion on Ni in food. The assessment of risks to the environment due to the presence of Ni in feed was confined to an estimation of its contribution to environmental Ni load as a result of its presence in manure.

1.2. Previous assessments

The assessments presented in the opinion on Ni in food (EFSA CONTAM Panel, 2015) have been reviewed and evaluated for the present opinion and, wherever appropriate, used as a starting point or taken over entirely for the present assessment. Wherever this has been done, it has been duly referenced.

An overview of relevant elements for risk assessment concerning exposure to Ni was provided by a Technical Report submitted to EFSA in 2010 (Van Paemel et al., 2010). This included a monograph

⁷ Commission Regulation (EU) No 68/2013 of 16 January 2013 on the Catalogue of feed materials. OJ L 29, 30.1.2013, p. 1–64.

on Ni, which contained key elements for risk assessment addressing the biological role, content in feed and requirements in animal nutrition based on the following publications:

- RIKILT (2008) assessed that the additional intake of Ni originating from mineral feed mixes to the human daily intake is marginal.
- The European Union Risk Assessment Report (EU RAR, 2008) reviewed the toxicological profile of Ni and Ni compounds in 2008 and concluded that there is no reason for concern for the general population not already sensitized to Ni following exposure to Ni metal or to Ni sulphate or chloride.
- In 2005, EFSA's Panel on Dietetic Products, Nutrition and Allergies in reply to a request of the European Commission related to the tolerable upper intake level of Ni, considered the available data to be inadequate to derive such a value (EFSA, 2005).
- In 2005, the Agency for Toxic Substances and Disease Registry (ATSDR, 2005) reviewed the toxicological profile of Ni and concluded that data were inadequate to derive an acute, intermediate or chronic minimum risk level (MRL).
- In 2005, the US National Research Council of the National Academies (NRC, 2005) concluded that the available data indicated that edible tissues and products do not contain enough Ni to be of toxicological concern for human beings.
- In 1993 and 2005, tolerable daily intake (TDI) values of 5 and 22 micrograms of Ni/kg body weight (b.w.) per day were established by the World Health Organization, respectively (WHO, 1993, 2005).
- In 2003, the UK Food Standards Agency (FSA) Group on Vitamins and Minerals concluded that a total Ni intake of 0.43 µg Ni/kg b.w. per day would not be expected to have effects in non-sensitised individuals (FSA, 2003).
- In 2001, the Institute of Medicine (IOM, 2001) found no evidence available for adverse effects associated with the exposure to Ni through the consumption of a normal diet and established an upper intake level for Ni of 1 mg/day for adults that applies to excess Ni intake as soluble salts.
- Tolerable daily intake (TDI) values of 50 micrograms of Ni/kg b.w. per day were proposed by the National Institute of Public Health and the Environment, the Netherlands (RIVM, 2001) and by Health Canada (1996).
- Reference Doses (RfD) values of 8 and 20 µg of Ni/kg b.w. per day were proposed, respectively by the US Environmental Protection Agency (US EPA, 2001) and by the Toxicology Excellence for Risk Assessment (TERA) in 1999.

More recently, the International Agency for Research on Cancer (IARC, 2012) classified Ni compounds as carcinogenic to humans (Group 1), and metallic Ni as possibly carcinogenic to humans (Group 2B). The carcinogenicity of Ni has been well documented in occupationally exposed individuals. Significant increases in the risk of mortality from lung and nasal cancers were observed in several cohorts of Ni refinery workers.

1.3. Chemistry

Ni is a silvery-white, hard, ductile metal and one of only few elemental metals that are magnetic at room temperature; bulk Ni is non-magnetic above approximately 350 °C (Curie point). The element has the basic physico-chemical properties described in Table 1.

Table 1: Some relevant physico-chemical properties of elemental nickel

Atomic number: 28	Boiling point: 2730 °C (3003 K)
Atomic mass: 58.69 amu	Vapour pressure: ≈ 1 Pa at 1728 K; 100 kPa at 3 186 K
Chemical family: transition metals, d-block Group 10 (VIII-B) of periodic table	Density: 8.908 g/cm ³ (at room temperature)
Electron configuration: [Ar] 4s ² 3d ⁸ or [Ar] 4s ¹ 3d ⁹	Solubility in water: practically insoluble
Electronegativity (Pauling scale): 1.91	Corrosion-resistant at room temperature. Reactive in air in powdered form, may spontaneously ignite. Dissolves readily in dilute mineral acids and aqua regia ^(a) but is passivated ^(b) by concentrated nitric acid. Highly resistant to attack by strong alkalis.
Melting point: 1455 °C (1728 K)	

(a): nitro-hydrochloric acid.

(b): addition of protective material to protect against corrosion.

The chemistry and physico-chemical properties of the transition metal Ni will not be extensively reviewed in this chapter especially since they have been recently summarized in the opinion on food (EFSA CONTAM Panel, 2015). This chapter only summarises the most important aspects regarding the chemistry of Ni in feed.

In general Ni can exist in the oxidation states -1 , 0 , $+1$, $+2$, $+3$, and $+4$, with the divalent oxidation state (Ni(II)) being the most relevant under normal conditions. In the Earth's surface Ni is found in different minerals in its divalent form, while in air Ni can occur in the form of particulate species, especially as NiO (CAS: 1313-99-1), NiS (CAS: 16812-54-7) and Ni₂S₃ (CAS: 12035-72-2). In aqueous biological systems Ni is naturally present in its divalent form as ion (Ni²⁺), complexed by or bound to biomolecules. In natural waters (pH range of 5–9) not containing strong complexing agents, aqueous Ni(II) occurs mostly as the hexaquaonickel ion [Ni(H₂O)₆]²⁺; complexes with common ligands – HCO₃[–], Cl[–], OH[–], NH₃, SO₄^{2–}, etc. – are formed to a minor degree. Ni is slightly more resistant to oxidation than iron and cobalt: its standard potential at 25 °C is -0.257 ± 0.008 V (Ni²⁺ + 2e[–] → Ni⁰) (Bard et al., 1985). Higher oxidation states of Ni are characterized by strong oxidative potentials and are not stable in water (US EPA, 1986; IARC, 2012). However, the formation of Ni(II)-Ni(III) redox couple in cells is one of the proposed mechanisms for the induction of free reactive species that cause oxidative stress as well as respective consequences *in vivo* (EFSA CONTAM Panel, 2015).

The natural occurrence of metallic Ni (Ni⁰) is extremely rare. Nevertheless, feed might contain metallic Ni, since it is used as a catalyst in the production of certain feed materials listed in the EU Catalogue of feed materials (Commission Regulation (EU) No 68/2013). These feed materials may contain Ni following hydrogenation of the fatty acids in which Ni is used as a catalyst. In the EU Catalogue it is expected that feed materials would not contain more than 50 mg/kg Ni when produced according to good manufacturing processes. Manufacturers are required to declare the Ni content of these feeds where it exceeds 20 mg/kg.

In most experimental studies investigating essential or toxic effects of Ni in plants or animals divalent Ni salts, NiCl₂ (CAS: 7718-54-9) and NiSO₄ (CAS: 7786-81-4) and their hydrated forms, have been used.

There are five naturally occurring stable Ni isotopes, with mass numbers 58 (68.07 %), 60 (26.23 %), 61 (1.14 %), 62 (3.63 %), and 64 (0.93 %). Several radioactive isotopes are also known: with the exception of ⁵⁹Ni and ⁶³Ni, whose half-lives are 76 000 and 100 years, respectively, they all exhibit short half-lives, in the order of a few days or, in general, much shorter. With the exception of ⁵⁹Ni, which is of cosmic origin, all the other radioactive isotopes have an artificial origin.

1.4. Sources and environmental fate

The environmental sources and fate of Ni are extensively discussed in the opinion on Ni in food (EFSA CONTAM Panel, 2015), with specific reference in air, in water bodies, in sediments and soils as well as in food. Therefore, the present section focusses on the presence of Ni in plant tissues.

Ni occurs naturally in soils and plants, and usually in concentrations substantially higher than those normally present in animal tissue and fluids.

Depending on local geology and anthropogenic input, levels of Ni in soil vary widely (ATSDR, 2005). In most agricultural soils Ni concentrations range from 3 to 1 000 mg/kg, (EFSA CONTAM Panel, 2015) but in polluted soils concentrations of up to 26 000 mg/kg have been reported (summarized in Yusuf et al., 2011). Ni can exist in oxidation forms -1 , 0 , $+1$, $+2$, $+3$ and $+4$, but in biological systems Ni (II) predominates. It occurs naturally in soils as a result of the weathering of the parent rock (McGrath, 1995). It is generally distributed uniformly through the soil profile, with the highest concentrations found in igneous rocks, and much lower levels found in sedimentary rocks (shales, clays, limestone, and sandstones). Atmospheric deposition of Ni has occurred as a result of the burning of oil and coal. Agricultural fertilisers, particularly phosphates, are also a significant source of Ni in soil but it is unlikely to build-up in soil in the long term from their use. The application of wastes including sewage sludge, to land has been reported to result in a build-up of Ni in soils and vegetation (Nicholson et al., 1999), although the extent to which this occurs is influenced by a number of factors, including the amount of sludge applied and its composition, and the parent soil type and composition. To guard against this build-up in soils, EU Sludge Directive 86/278/EEC⁸ (as amended) sets maximum permitted concentrations, in both sludge and soil, of a range of heavy metals including Ni. The use of sewage sludge is prohibited if the concentration of Ni in soil exceeds 75 mg/kg dry matter (DM). In the United Kingdom (UK), it has been estimated that approximately 210 tonnes of Ni are deposited onto agricultural land annually, equivalent to approximately 230 g/ha. Of this, sewage sludge (20 %), other composts (15 %) and livestock manures (10 %) were the main contributors (Nicholson et al., 2010). Of the livestock manures, 77 % of deposited Ni was from cattle, with lesser amounts from poultry (13 %), pigs (7 %) and sheep (3 %).

The uptake of Ni from soil in plants is through the root system via both passive diffusion and active transport (Seregin and Kozhevnikova, 2006). It is absorbed predominantly in the form of the Ni^{2+} ion, and relative ratios of uptake (active and passive transport) vary with the species, the form of the Ni and concentration in the soil, nutrient solution or water, plant metabolism, soil acidity and the presence of other metals and organic matter (Chen et al., 2009). Ni uptake declines as soil pH increases due to the formation of less soluble complexes (Yusuf et al., 2011). The Ni^{2+} ion competes with other essential metal ions when absorbed by roots, and is strongly affected by calcium ion Ca^{2+} . Following absorption, Ni is transported through the plant via the transpiration stream in the xylem, with organic acids and amino acids acting as chelators to facilitate transport in the xylem (Yusuf et al., 2011). It is stored principally in the shoots and leaves during vegetative growth (Cataldo et al., 1978). Available evidence about possible absorption of Ni through leaves is limited and controversial, although applying Ni in foliar sprays has been recommended in some situations where Ni deficiency has been identified.

Although Ni is an essential nutrient for higher plants, the amount required for normal growth is very low. Dixon et al. (1975) first identified the role of Ni^{2+} for urease activation, and Ni^{2+} was subsequently determined to be essential in higher plants by several authors (Eskew et al., 1984; Brown et al., 1987; Marschner, 1995). However, at toxic levels Ni disrupts nutrient uptake by the roots and photosynthesis (Chen et al., 2009). As noted by Chaney (1990), the low tolerance by plants to excess Ni effectively acts as a protective barrier to livestock. While reductions in yield of 25 % or more occur in most plants when Ni concentrations (in the leaves) exceeds 10 mg/kg in sensitive plants or

⁸ Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. OJ L 181, 4.7.1986, p. 6–12.

50 mg/kg DM in moderately tolerant ones, cattle can usually tolerate levels of up to 200 mg/kg in their diet for extended periods without adverse effects.

Although Ni is usually present in feedingstuffs, the form of Ni in foods and feeds is generally not determined (NRC, 2005).

2. Legislation

Council Directive 2002/32/EC regulates undesirable substances in products intended for animal feed. Annex I to this regulation contains a list with MLs for certain inorganic and organic feed contaminants, but Ni is not considered in this Directive. Commission Regulation 68/2013 stipulates that, in accordance with Article 4 of Regulation (EC) 183/2005,⁹ which refers to good practice in feed production that feed materials shall be free from chemical impurities resulting from their manufacturing process, unless a specific maximum content is fixed in the EU Catalogue of feed materials. This Catalogue is contained in the Annex to this Regulation. Part C of the Catalogue presents a list of feed materials. Ni is listed as being present in certain feeds as a result of the manufacturing process in several feed materials. Fatty acids esterified with glycerol (Catalogue number 13.6.2) crude fatty acids from splitting (mono-, di- and tri-glycerides of fatty acids (13.6.3), fatty acid distillates (13.6.5), crude fatty acids (13.6.6) and pure distilled fatty acids (13.6.7) may contain up to a maximum of 50 mg/kg Ni, which is considered as a level achievable by applying good manufacturing practice. Any content higher than 20 mg/kg needs to be declared.

There are currently no maximum levels in the EU legislation for Ni in food. There is also no regulatory limit for release of Ni from food contact materials in the EU.

Regarding drinking water, a value of 20 µg/L for Ni is included in the EU Council Directive 98/83/EC¹⁰ 'on the quality of water intended for human consumption', and a maximum limit of 20 µg Ni/L is set by the Commission Directive 2003/40/EC for natural mineral waters.

Within the EU, the application of sewage sludge to land is regulated by the Sewage Sludge Directive (86/278/EEC). While this seeks to encourage the use of sewage sludge in agriculture, it prohibits the use of untreated sludge on agricultural land unless it is injected or incorporated into the soil. The Directive sets maximum levels for heavy metals in both the sludge and the soil to which it is applied, and the use of sewage sludge is prohibited if the concentration of Ni in soil exceeds 75 mg/kg DM. In order to provide protection against potential health risks from residual pathogens, Directive 86/278/EEC states that grazing animals must not be allowed access to grassland or forage land less than three weeks after the application of sludge.

3. Methods of analysis

3.1. Sampling and storage

There are no specific guidelines for the sampling of feed to be analysed for their total Ni content. Therefore, basic rules for sampling of trace elements should be followed. The primary objective is to obtain a representative and homogeneous laboratory sample with no secondary contamination during sample preparation. Wherever possible, apparatus and equipment that come into contact with the sample should not contain Ni and should be made of inert materials, e.g. titanium or ceramic knives, agate mortar or ball mill for size reduction and homogenisation instead of stainless steel or iron equipment. These should be acid cleaned to minimise the risk of contamination. Methods of sampling

⁹ Regulation (EC) No 183/2005 of the European Parliament and the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1–22.

¹⁰ Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p. 1–28.

and analysis of feed in general are given in Commission Regulation (EC) No 152/2009.¹¹ Therefore, the sampling rules as laid down in the Regulation should be followed.

3.2. Instrumental techniques

3.2.1. Total nickel analysis

The methods of analysis of total Ni in environmental samples have been reviewed by ATSDR (2005). Prior to analysis, feed samples have to be homogenized and digested. The most common methods used to detect Ni in feed are atomic absorption spectrometry (AAS), either flame or graphite furnace (FAAS, GFAAS), inductively coupled plasma optical emission or mass spectrometry (ICP-OES or ICP-MS).

3.2.2. Metallic nickel analysis

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) did not identify any methods for the quantification of metallic Ni and soluble Ni species in feed. In airborne particulate matter total Ni and Ni species fractions (soluble Ni, sulphidic Ni, metallic Ni and oxidic Ni) are quantified after a sequential extraction procedure by AAS or ICP-MS (Schaumlöffel, 2012). After optimisation, this combination of extraction and elemental quantification might also be applicable to distinguish between metallic and soluble Ni in feed samples.

4. Occurrence of nickel in feed

4.1. Previously reported occurrence data on nickel in feed

Published occurrence data on the Ni concentrations in animal feed and feed materials are limited. Nicholson et al. (1999) reported the Ni content in 183 livestock feeds from commercial farms in England and Wales. Ni concentrations in dairy cattle feed and beef cattle feed ranged from 0.1 to 11.2 mg/kg DM and from 0.2 to 8.3 mg/kg DM, respectively, depending on the feed type and are presented in Table 2. Alexieva et al. (2007) reported Ni levels in feed materials in a larger range, 0–16 mg/kg, however this was not on a dry matter basis.

Table 2: Nickel (Ni) concentrations in different types of feed materials

Feed type	N	DM	Ni	
		Mean %	Mean mg/kg DM	Range mg/kg DM
Dairy cattle feed ^(a)				
Dairy cake/nuts	15	86.2	2.8	0.6–7.2
Maize gluten	6	86.2	1.6	0.8–3.6
Molasses	4	63.9	1.2	0.3–2.1
Sugar beet pulp	3	87	2.5	1.3–4.0
Minerals	5	96.7	9.0	7.3–11.2
Cereals	1	86.2	<1.0	–
Grass silage	18	28.9	0.8	0.1–2.0
Maize silage	2	28.6	4.3	0.4–8.2
Beef cattle feed ^(a)				
Beef cake/nuts/pellets	9	86.3	3.1	2.1–4.3
Rolled oats and barley	4	84.1	2.4	0.3–8.3
Hay	2	83.0	0.8	0.5–1.1
Straw	4	88.7	0.5	0.3–0.7
Grass silage	10	35	1.1	0.2–2.5

¹¹ Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. OJ L 54, 26.2.2009, p. 1–130.

Feed type	N	DM	Ni	
		Mean %	Mean mg/kg DM	Range mg/kg DM
Feed materials ^(b)				
Wheat	16	—	2.2	0–14
Corn	17	—	1.0	0–1.5
Barley	10	—	0.5	0–1
Wheat bran	8	—	1.8	1–2.8
Sunflower meal	12	—	7.8	5–9.5
Soybean meal	17	—	3.9	2–6.8
Fishmeal	5	—	2.2	1.5–3
Other ingredients	8	—	5.7	0–16

DM: dry matter; N: number of samples.

(a): Nicholson et al. (1999).

(b): Alexieva et al. (2007).

The Ni concentrations in compound feeds reported in a number of studies are given in Table 3. Compound feeds for pigs contained between 0.4–4.3 mg/kg DM and 1.3–6.8 mg/kg fresh weight from England and Wales, and Bulgaria, respectively. In poultry feeds, Ni concentrations ranged from 1.1 mg/kg (DM) to 7.0 mg/kg (fresh weight), with higher levels reported in feeds from Bulgaria (Alexieva et al., 2007) compared with England and Wales (Nicholson et al., 1999). Ni levels in poultry feed samples used as starter, grower, developer, layer, rabbit feed and bran from Saudi Arabia ranged from 0.45 to 3.26 mg/kg (Alkhalaf et al., 2010).

Table 3: Nickel (Ni) concentrations in different types of compound feed

Feed type	N	DM	Ni	
		Mean %	Mean mg/kg DM	Range mg/kg DM
Compound feed for pigs				
Rearer - creep ^(a)	4	89.8	2.3	2.2–2.6
Rearer - weaner ^(a)	4	87.7	2.3	2.0–2.6
Rearer - grower ^(a)	5	87.8	3.1	1.7–3.5
Rearer - finisher ^(a)	7	87.9	2.8	1.2–4.3
Sow - dry ^(a)	3	86.9	2.7	2.0–3.7
Sow - lactating ^(a)	3	87.7	1.2	0.4–2.1
Pigs ^(b)	30	—	3.2*	1.3–6.8*
Compound feed for poultry				
Broiler - starter ^(a)	4	88.8	2.0	1.1–2.8
Broiler - grower ^(a)	4	88.5	2.0	1.3–2.8
Broiler - finisher ^(a)	3	88.5	2.1	1.1–3.9
Turkey - various ^(a)	6	88.6	1.8	0.7–2.8
Turkey - grower ^(a)	4	87.2	2.0	0.8–3.0
Turkey - finisher ^(a)	3	87.3	1.7	0.9–2.2
Layer ^(a)	4	89.0	2.6	1.3–5.2
Poultry - layers ^(b)	12	—	5.3*	3.8–7.0*
Poultry - broilers and pullets ^(b)	17	—	3.6*	3.0–4.5*

DM: dry matter; N: number of samples; *: results given on a fresh weight basis and not as DM.

(a): Nicholson et al. (1999).

(b): Alexieva et al. (2007).

Few data are available for Ni levels in complete fish feed. Commercial fish feed samples from six manufacturers collected from 11 commercial fish hatcheries in the US were analysed for various chemical constituents including Ni (Maule et al., 2007). The mean moisture content in the feeds varied considerably among the different hatcheries from 6.6 to 42.8 %, and were high compared to the typical moisture content of commercial, pelleted fish feed (e.g. 4–9 % moisture (Oehme et al., 2014). Ni

concentrations in commercial fish feed are presented in Table 4 (the minimum and maximum Ni concentrations in fish feed from all six manufacturers were 0.5–9.3 mg/kg, respectively).

Table 4: Nickel (Ni) concentrations (mean and SD, mg/kg) in different types of complete fish feed

Feed manufacturer	N	DM	Ni	
		Mean %	Mean mg/kg DM	SD mg/kg DM
A	7	77.2	3.2	1.1
B	10	93.4	3.0	0.7
C	4	57.2	3.6	1.0
D	15	92.1	2.8	0.8
E	15	91.8	2.8	0.8
F	4	92.5	2.6	0.8
Total	55	84.0	2.8	1.7

DM: dry matter; N: number of samples; SD: standard deviation.

Relatively few data have been published on the Ni content of forages. Surveys from different countries (Kabata-Pendias and Pendias, 1992) reported mean levels of 0.13–1.1 mg/kg and 1.2–2.7 mg/kg for pasture grasses and legumes, respectively. As discussed elsewhere (Sections 1.4, 2, 6.4) sewage sludge is widely used as agricultural manure, and may be applied to land on which livestock graze. A number of studies have examined the effects of applying sewage sludge to Ni concentrations in herbage. Fitzgerald et al. (1985) studied the build-up of Ni (and other heavy metals) following the application of anaerobically digested sewage sludge to grassland between 1975 and 1978. Mean Ni concentrations in irrigated plants ranged from 8 to 19 µg/kg, compared to 2–8 µg/kg in non-irrigated plants. Over a longer period (1985–1993) Aitken and Cummins (1997) reported an increase in soil Ni concentrations, as a result of applying sewage sludge to grassland in Lanarkshire (UK), but no increase in plant Ni content. The authors speculated that this response was mediated, in part at least, by soil pH since increasing the soil pH from 5.5 to 6.5 decreased plant uptake of Ni.

4.2. Current occurrence results

4.2.1. Data collection summary

By the end of April 2014, a set of 1 699 analytical data on Ni in feed were available in the EFSA database. The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description for Food and Feed (EFSA, 2010a). All analytical data were reported as Ni, without mention of specific chemical species; results were expressed as whole weight. A group of 114 samples, initially codified as ‘grain as crops’ and for which their final end-use was undefined, were considered as feed. They corresponded to 19 samples of wheat grain and 95 samples of barley grain. Therefore, the final dataset contained a total of 1 813 analytical data on Ni in feed.

The data for the present assessment were provided by the national authorities of Slovakia and Finland within the framework of the annual data collection. Most of the samples were collected in just one country, Slovakia (1 794 samples), with 19 samples collected in Finland. Analytical data reported were on samples collected between 2007 and 2011 and showed a similar distribution.

In order to guarantee an appropriate quality of the data used in the exposure assessment the initial dataset was evaluated, searching for incomplete or incorrect description of the relevant variables (e.g. parameter type, food classification, result value, limit of detection (LOD) or limit of quantification (LOQ)). The data set was also checked for duplicates (same sample transmitted twice or repeated analysis of the same sample). No samples were excluded.

4.2.2. Distribution of samples across feed categories

According to Commission Regulation No 68/2013 classification, the available feed samples belonged to six different groups, namely ‘Cereal grains and products derived thereof’, ‘Oil seeds, oil fruits, and

products derived thereof', 'Legume seeds and products derived thereof', 'Tuber, roots, and products derived thereof', 'Forage and roughage, and products derived thereof', and 'Compound feed' (Figure 1). The most represented feed groups were 'Cereal grains and products derived thereof' and 'Forage and roughage, and products derived thereof', with 579 and 710 samples reported, respectively. Among the feed group 'Cereal grains and products derived thereof' the most represented samples were those of maize ($n = 168$), barley ($n = 167$) and wheat ($n = 156$). The feed group 'Forage and roughage, and products derived thereof' was mainly represented by samples of forage meal ($n = 524$) and lucerne ($n = 118$). It is also worth mentioning the presence of 320 samples of 'Compound feed', with 317 reported as complete feed and three as complementary feed (Table 5); among the samples of complete feed all but three were reported as unspecified with respect to the livestock category for which they were intended.

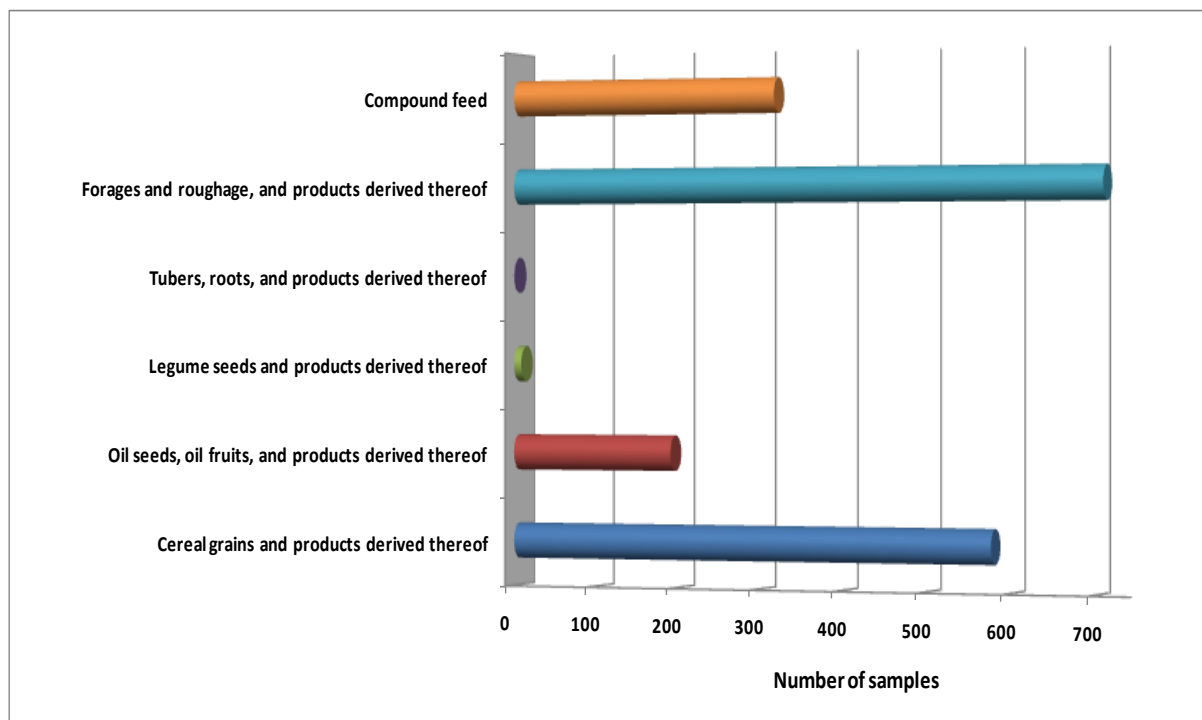


Figure 1: Distribution of feed samples for nickel (Ni) across the feed categories according to Commission Regulation No 68/2013

4.2.3. Analytical methods

Two different analytical methods were reported for the 1 813 analytical results available on total Ni, atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS). Limits of detection (LODs) and limits of quantification (LOQs) were both reported for all observations.

The most reported method was AAS, used for 1 671 samples by three different laboratories. With the exception of three samples of compound feed for which high LOD and LOQ were reported (70 and 200 $\mu\text{g/kg}$, respectively), the LODs varied between 1 $\mu\text{g/kg}$ and 20 $\mu\text{g/kg}$, while the LOQs ranged between 4 $\mu\text{g/kg}$ and 50 $\mu\text{g/kg}$. For ICP-MS, only 142 analytical results were reported by two laboratories. Reported LODs were 2 $\mu\text{g/kg}$ and 6.7 $\mu\text{g/kg}$, while LOQs were 5 $\mu\text{g/kg}$ and 20 $\mu\text{g/kg}$.

4.2.4. Occurrence data by feed category

All results were expressed as whole weight, without reporting information on the moisture content. Only 6 % (111 samples) of the analytical data were left-censored concentrated in the feed groups 'Cereal grains and products derived thereof' and 'Forage and roughage, and products derived thereof' (53 and 43 left-censored data, respectively).

The left-censored data were treated by the substitution method as indicated in the EFSA scientific report ‘Management of left-censored data in dietary exposure assessment of chemical substances’ (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the LB and UB approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). At the LB, results below the LOQ were replaced by zero; at the UB the results below the LOQ were replaced by the value reported as LOQ.

Table 5 shows a detailed description of the available feed samples with the summary statistics on their levels of Ni. In this table, the samples are grouped as they were used to calculate the animal exposure. The minimum mean Ni concentration among the different feed groups was reported in rye ($n = 7$, LB = 138.6 $\mu\text{g/kg}$, UB = 141.4 $\mu\text{g/kg}$) while the maximum mean value was reported in samples of toasted soya beans ($n = 9$, LB = UB = 3 895.2 $\mu\text{g/kg}$). The concentration of Ni among the individual quantified samples ranged between 4 $\mu\text{g/kg}$ in a sample of barley and 11 300 $\mu\text{g/kg}$ in a sample of toasted soya bean.

Within the feed group ‘Cereal grains and products derived thereof’ the highest mean levels of Ni were reported in the six samples of unspecified cereal grains and products derived thereof (LB = UB = 771.3 $\mu\text{g/kg}$), and in oat grains (LB = UB = 1 172.8 $\mu\text{g/kg}$, $n = 24$). The highest mean values for the feed group ‘Forage and roughage, and products derived thereof’ were also reported in unspecified samples (LB = UB = 1 636.9 $\mu\text{g/kg}$, $n = 17$), and in samples of forage meal (LB = 650.8 $\mu\text{g/kg}$, UB = 651.5 $\mu\text{g/kg}$, $n = 524$). Apart from oat grains, the cereal grains possessed relatively low values of Ni, with the lowest mean concentrations reported for rye, as mentioned above, and for barley (LB = 193.5 $\mu\text{g/kg}$, UB = 195.1 $\mu\text{g/kg}$, $n = 195$), and the highest for maize (LB = 406.7 $\mu\text{g/kg}$, UB = 407.4 $\mu\text{g/kg}$, $n = 173$). Across the samples of ‘Compound feed’ relatively high values of Ni were found, with mean values of 1027.5 $\mu\text{g/kg}$ at the LB and 1 028.0 $\mu\text{g/kg}$ at the UB reported for the samples of complete feed ($n = 317$). These values are in line with previously reported occurrence data on different types of compound feed (see Table 3 above).

Table 5: Feed samples with available data on nickel (Ni) ($\mu\text{g/kg}$) classified according to the Catalogue of feed materials specified in Commission Regulation (EU) No 68/2013

Feed material groups	Feed materials	N ^(a)	LC ^(b)	LB/UB	Mean ^(c)	Percentiles ^(d)				
						5th	25th	Median	75th	95th
Cereal grains and products derived thereof	Cereal grains, their products and by-products	6	–	LB	771.3	–	–	375.0	–	–
				UB	771.3	–	–	375.0	–	–
	Barley	195	28	LB	193.5	0.0	50.0	130.0	250.0	479.0
				UB	195.1	10.0	50.0	130.0	250.0	479.0
	Wheat	174	18	LB	399.8	0.0	100.0	213.5	440.0	1716.0
				UB	401.3	10.0	100.0	213.5	440.0	1716.0
	Maize	173	5	LB	406.7	28.0	90.0	220.0	489.0	1520.0
				UB	407.4	30.0	90.0	220.0	489.0	1520.0
	Oats	24	–	LB	1 172.8	–	165.0	1090.0	1789.5	–
				UB	1 172.8	–	165.0	1090.0	1789.5	–
Oil seeds, oil fruits, and products derived thereof	Rape seed	146	4	LB	745.4	–	240.0	512.5	948.0	–
				UB	745.7	–	240.0	512.5	948.0	–
	Toasted soya (beans)	9	–	LB	3895.2	–	–	3021.0	–	–
				UB	3895.2	–	–	3021.0	–	–
	Sunflower seed	39	–	LB	1566.3	560.0	1070.0	1370.0	1850.0	4001.0
				UB	1566.3	560.0	1070.0	1370.0	1850.0	4001.0
Legume seeds and	Legume seeds and	9	–	LB	1125.0	–	–	1150.0	–	–

Feed material groups	Feed materials	N ^(a)	LC ^(b)	LB/UB	Mean ^(c)	Percentiles ^(d)				
						5th	25th	Median	75th	95th
products derived thereof	products derived thereof			UB	1125.0	–	–	1150.0	–	–
Tubers, roots, and products derived thereof	Potatoes	1	–	LB	207.0	–	–	–	–	–
				UB	207.0	–	–	–	–	–
Forages and roughage, and products derived thereof	Forages and roughage, and products derived thereof	17	–	LB	1636.9	–	770.0	1170.0	2020.0	–
				UB	1636.9	–	770.0	1170.0	2020.0	–
	Lucerne; [Alfalfa]	118	4	LB	1166.3	40.0	150.0	625.0	1877.0	3720.0
				UB	1166.7	40.0	150.0	625.0	1877.0	3720.0
	Cereals straw	1	–	LB	1111.0	–	–	–	–	–
				UB	1111.0	–	–	–	–	–
	Clover meal	50	5	LB	483.4	0.0	50.0	210.0	570.0	
				UB	484.4	10.0	50.0	210.0	570.0	
	Forage meal; [Grass meal]	524	34	LB	650.8	0.0	140.0	352.0	751.5	2363.0
				UB	651.5	10.0	140.0	352.0	751.5	2363.0
Compound feed	Complete feed	317	11	LB	1027.5	40.0	360.0	740.0	1480.0	2985.0
				UB	1028.0	40.0	360.0	740.0	1480.0	2985.0
	Complement ary feed			LB	1260.0	–	–	–	–	–
	(incomplete diet)	3	–	UB	1260.0	–	–	–	–	–

LB: lower bound; LC: left-censored; UB: upper bound.

(a): Number of available samples;

(b): Number of left censored data;

(c): Mean value expressed as µg/kg fresh weight. Feed groups with exactly the same result as mean value denotes the absence of left-censored (unquantified) data.

(d): The different percentiles were only described when a minimum number of samples were available, 60 samples for 5th and 95th percentiles, 11 samples for 25th and 75th percentiles, and 6 samples for the median. Otherwise, the percentiles may not be statistically robust.

4.3. Feed processing

For many livestock, vegetable oils are important feed ingredients. Following extraction, the oils may be hydrogenated, which has the effect of improving handling characteristics of the oil and reducing the risk of oxidation, thereby improving the keeping qualities of the oil (Allen, 1978). Ni is used as the catalyst, and is present as both metallic Ni and Ni oxide. Once the desired degree of hydrogenation has been achieved, the hydrogen flow is stopped and the catalyst is filtered from hydrogenated oil. According to Venne (1993) virtually all the spent Ni catalyst is recovered and reused, although trace amounts may remain.

In February 2015 the European Vegetable Oil and Protein Meal Industry Federation (FEDIOL) provided EFSA with data on the content of Ni in 665 hydrogenated vegetable oils, analysed in the EU between 2012 and 2015, (Julie Roiz, 2015, FEDIOL personal communication). The median Ni content reported was 0.12 mg/kg and the maximum reported level was 17 mg/kg, and therefore none of the samples had Ni contents that exceeded the level (20 mg/kg) that requires it to be declared (Commission Regulation (EU) No 68/2013). However, since these data were not available within the timeframe of this mandate, data validation could not be carried out and therefore they data were not used to estimate animal exposure.

Stainless steel materials are widely used for food and feed processing equipment and containers. Although the quality of stainless steel used is usually selected to adequately meet the varying requirements of corrosion resistance in the different foods or feeds being processed, small amounts of metallic elements in the stainless steel may migrate into the food or feed being processed. The extent to which this may occur is influenced by both the quality of the stainless steel and the composition – particularly the pH – of the material being processed. A number of countries including France, Italy, Germany and the UK have their own national standards for stainless steel quality, although these are being superseded by the ISO standards. In addition, there are European Standards for certain types of food contact application of stainless steels.

Ni migration as a result of food processing may be a source of dietary exposure to Ni. However, studies on the migration of Ni from cooking utensils made of ferritic and austenitic stainless steel suggest that its contribution to an average daily diet for humans is negligible compared to the contribution from Ni naturally present in food.¹² No data have been identified for feed processing, but the CONTAM Panel assumes that Ni migration may be similar with respect to processing of animal feeds.

5. Feed and water consumption

5.1. Feed consumption

A wide range of feeds is used in the diets of livestock and companion animals in the EU; the EU Catalogue of feed materials lists over 600 different feeds, although the majority can be broadly classified as cereal grains and their by-products, oilseed meals and their products, and the by-products of food production and forages.

Grains and their by-products are widely used as feed for livestock; in the EU more than 74 million tonnes are used in manufacture of compound feeds, accounting for 48 % of all feed materials used,¹³ almost all of which (> 95 %) are grown or produced in the EU. In addition to incorporation in compound feeds, cereal grains and by-products are frequently fed in on-farm mixes or as single ingredients, particularly to supplement forages for ruminant livestock. Therefore, the total amount of cereal grains and cereal by-products used as feed for livestock will be considerably greater than that reported for compound feed production. However, there are no data on the total amounts of cereals used as feed, either by type (wheat, barley, etc.) or by livestock species (cattle, pigs, poultry, etc.).

Oilseed meals are also widely used in livestock feeds, and in 2012 accounted for 28 % of all non-forage feed materials consumed in the EU. Just over 50 % are soybean and soybean meal, almost all of which is imported. Rapeseed and sunflower seeds and meals account for 30 % of protein-rich feed materials used, and most of these are produced in the EU. Again, there are no publicly available data on the types of livestock to which these are fed. By-products from the food industry account for a further 11 % of non-forage feeds used.

In addition to these major feed materials, diets for livestock are supplemented with trace elements and vitamins.¹⁴ These are usually provided as mixtures appropriate to the physiological needs of the livestock, e.g. for pregnancy or lactation, lean tissue growth or egg production and usually form part of the compound feed or complementary feed.

It is also common practice to include vegetable oils in rations for livestock. The type and amounts added will vary depending on the animal species and other feeds being used, but usually will not exceed about 5 %. For some of the feed materials in this category, specifically for products obtained

¹² Council of Europe's Policy Statements Concerning Materials and articles intended to come into contact with foodstuffs - policy statement concerning metals and alloys – technical document – guidelines on metals and alloys used as food contact materials – 13.02.2002: www.coe.int/soc-sp

¹³ Source: European Food Manufacturers Federation – FEFAC (<http://www.fefac.eu>).

¹⁴ Defined under EU legislation as feed additives and controlled by Regulation (EC) No 1831/2003 of the European Parliament and the Council of 22 October 2003 laying down requirements for feed hygiene. OJ L35, 8.2.2005, p. 1–22.

by hydrogenation (fatty acids, salts of fatty acids, crude fats obtained from splitting and glycerine), Commission Regulation (EU) 68/2013 states that these may contain up to 50 mg/kg. If levels exceed 20 mg/kg, then the amount has to be declared. For non-ruminants, digestibility of hydrogenated oils tends to be lower than for unhydrogenated oils, and therefore they are not widely used in rations for pigs and poultry. In ruminants, unhydrogenated oils can adversely affect rumen digestion, and therefore hydrogenated oils tend to be more widely used in cattle and sheep diets.

As illustrated in Table 5, Ni occurs regularly in all feed materials, and therefore forms part of all livestock diets. Estimating the intake of Ni (Section 6) requires estimates of both the amount of feed consumed and the level of Ni in the feed. Estimates of feed intake by livestock, also expressed in terms of amount of feed/kg b.w. per day, are presented with details given in Appendix A. It should be stressed that the diets used in estimating Ni intake do not necessarily represent ‘average’ diets, nor are the feeding systems ‘typical’ for all of Europe. Instead, they are used to estimate levels of exposure to Ni that might not be atypical. They are based on published guidelines on nutrition and feeding (AFRC, 1993; Carabano and Piquer, 1998; NRC, 2007a,b; Leeson and Summers, 2009; McDonald et al., 2011; EFSA FEEDAP Panel, 2012), data on EU manufacture of compound feeds¹⁵ and expert knowledge of production systems in Europe.

5.1.1. Ruminants and horses

For most ruminants and horses, forages – either fresh or conserved – are the main ingredients in their diet, and in some cases may be the only feed available. For more productive livestock, forages are supplemented with additional feeds, usually cereal grains, oilseed meals and other by-products derived from food production, together with minerals and vitamins as required.

5.1.1.1. Dairy cows

For most dairy cows in the EU, forages are supplemented with cereal grains, cereal by-products and oilseed meals. In Northern Europe, barley and wheat are most commonly used, while in Southern Europe maize (corn) is more widely used. Because of their lower energy content relative to other cereals, oats are less widely used in diets for dairy cows. The amounts fed are adjusted according to the quality of the forage available and the milk yield of the cow, and adjusted for pregnancy and live weight gain, but will typically be about 0.25–0.35 kg feed/kg milk production (Nix, 2014).

In Section 6, exposure is estimated for a 650 kg dairy cow, with a milk yield of 40 kg/day. Assumptions on the proportions of forages and non-forage feed, and the proportions of cereal grains, their products and their by-products in the diet, are given in Appendix A1, Table A1.

5.1.1.2. Beef cattle

There are a wide variety of beef production and husbandry systems in Europe. They may be categorised broadly as pasture-based and cereal-based systems, although combinations of these systems are commonly found. For forage-based systems, the objective is usually to produce as much meat from minimal inputs of cereals, with levels typically less than 10 % of the total DM of feed consumed.

In contrast, intensive cereal-based systems of beef production provide diets that consist almost entirely of rolled cereal grains, supplemented with vegetable proteins (McDonald et al., 2011). In this system of production, feed intake may be as high as 2.5 % of the body weight (NRC, 2000). For the estimates of exposure (Section 6) it has been assumed that a 400 kg bull consumes 8.4 kg of DM feed per day (AFRC, 1993), of which 85 % is non-forage feed containing 75 % cereal grains, their products and by-products (Appendix A1, Table A1).

¹⁵ <http://www.fefac.eu>

5.1.1.3. Sheep and goats

As for other ruminants, good quality forage is the most important dietary ingredient and for many of these livestock forages may be the only feeds used after weaning (NRC, 2007a). Exceptions to this are pregnant and lactating animals, where supplementation with non-forage feeds or commercial compound feeds usually occurs (AFRC, 1993; NRC, 2007a). In addition, lambs and kids are frequently fed some compound feeds around the time of weaning to encourage the intake of solid feed.

The diets of sheep and goats reared for meat production consist predominantly of forage, with additional non-forage feeds given when high levels of live weight gain are required. Total daily DM intakes can range from 1.9 to 3.8 % of their body weight (Devendra and Burns, 1983), of which forages typically account for 75 % or more of total intake. Goats reared for meat production and with a body weight > 10 kg are often fed green fodder *ad libitum* (AFRC, 1993) supplemented with cereal grains (barley, oats or maize), cereal by-products and vegetable proteins (McDonald et al., 2011).

For milking sheep and goats, compound feeding usually commences in late pregnancy and continues into lactation, with the amounts fed depending on the quality and quantity of forage available and the stage of lactation. Non-lactating animals usually receive only forage feeds until just prior to parturition.

The feed intakes and diet compositions assumed by the CONTAM Panel to estimate exposure to Ni by milking sheep and goats are given in Appendix B, Table B1.

The CONTAM Panel have used daily DM intakes of 3.3 kg for a 60 kg goat for milking (4 kg milk/day) and 1.5 kg for a 40 kg goat for fattening to estimate the exposures to Ni. Details on the composition of the diets used in estimating the exposure for goats are given in Appendix B, Table B1.

5.1.1.4. Horses

Horses are complete herbivores. They will generally consume 2–3.5 % of their body weight in feed (DM) each day, of which a minimum of 50 % should be as forage (pasture or hay) (NRC, 2007b). Mature horses with minimal activity can be fed forage alone, but for growing and active horses supplementary feeding with cereal grains, cereal by-products (e.g. oats, barley, and wheat bran) and vegetable proteins is necessary. Although oats are the preferred cereals for many horse owners, other cereal grains and cereal by-products are also routinely used. Vegetable oils are not extensively used in horse feed. The CONTAM Panel estimated the exposure for a 450 kg horse, with a daily intake of 9 kg DM/day, of which half is in the form of forages and where cereal grains, their products and by-products represent 82 % of the non-forage component of the daily ration (Appendix A1, Table A1).

5.1.2. Pigs

There is a considerable range of diets fed to pigs in Europe, but they consist predominantly of cereals and cereal by-products, supplemented with vegetable proteins (e.g. soybean meal, peas and beans, rapeseed meal), minerals and vitamins. The relative proportions of these ingredients in the diets will vary depending on the quality of the feeds available and the nutritional needs of the animals. Diets for breeding pigs also tend to include greater proportions of fibrous feeds such as cereal by-products and sugar beet pulp (McDonald et al., 2011). Hydrogenated vegetable oils are not normally included in pig feed.

Exposure estimates have been made for piglets (20 kg b.w.), fattening pigs (100 kg b.w.) and lactating sows (200 kg b.w.) using feed intakes proposed by EFSA FEEDAP Panel (2012) (Appendix A1, Table A2). The proportions of cereal grains, their products and by-products used in estimating the exposure for pigs are given in Appendix A1, Table A2.

5.1.3. Poultry

Poultry have limited ability to digest fibre,¹⁶ and therefore cereal grains form the major part of their diets. In Europe, wheat, maize and barley are most commonly used, with rye, sorghum triticales and oats used less widely. Other ingredients include cereal by-products and vegetable proteins, supplemented with minerals, trace elements and vitamins (Leeson and Summers, 2009; McDonald et al., 2011). In order to increase the energy density of the diet and thus productivity, vegetable oils are frequently included in compound feeds for poultry although the hydrogenated forms are not widely used.

The amount of feed voluntarily consumed is largely determined by the size and age of the bird. Under *ad libitum* feeding daily intake increases as the birds get older, although relative to body weight it declines with age. For meat producing and egg laying birds, *ad libitum* feeding is widely practiced, but for breeding stock feed intake is frequently restricted to maintain a steady body weight (Leeson and Summers, 2009).

The CONTAM Panel applied the live weights and feed intakes reported for different poultry (broilers, laying hens and turkeys) by EFSA FEEDAP Panel (2012) and for ducks by Leeson and Summers (2009) for the exposure estimations (Appendix A1, Table A1).

5.1.4. Rabbits

Commercial rabbit production takes place in at least 14 EU Member States, but the largest producers are Italy, France and Spain. The EU is responsible for about 0.5 million tonnes, representing 55 % of world rabbit meat production. Commercial rabbit meat production in the EU is about 500 000 tonnes, corresponding to 100 million animals/year.¹⁷

Rabbits are usually fed a pelleted diet of dried forages, cereals and vegetable proteins supplemented with minerals, vitamins and trace elements. Lebas and Renouf (2009) reviewed diet formulations used in experimental studies: in 58 diets, cereals and cereal by-products (mostly wheat bran) accounted for up to 40 % of all ingredients. In these studies, maize was a major cereal grain and was included in more than one-third of all diets. In Northern Europe, however, maize may be replaced by barley and wheat. Feed intakes of 65–80 g/kg b.w. per day have been reported (Carabano and Piquer, 1998). For the exposure estimates, the CONTAM Panel assumed a live weight of 2 kg, and a feed intake of 75 g/kg b.w. per day. The proportions of cereal grains, their products and by-products used in estimating the exposure are given in Appendix A1, Table A2.

5.1.5. Farmed fish

Atlantic salmon is economically the most important farmed fish in Europe, although other commercially reared species include rainbow trout, sea bass, sea bream, cod, halibut, tuna, eel and turbot. Traditionally, the principal raw materials used for the manufacture of fish feeds in Europe have been fishmeal and fish oils, and although alternative sources of oil and protein (e.g. soybean meals and vegetable oils) are increasingly being used fish-derived feeds still remain the major ingredients. Details of the diet used to estimate exposure are given in Appendix A1, Table A3.

5.1.6. Dogs and cats

Most small companion animals derive their nutritional needs from processed food, and in 2010 EU annual sales of pet food products was approximately 8.3 million tonnes.¹⁸ Although a wide range of ingredients is used in commercial diets, most dog and cat diets contain at least some animal protein. Other ingredients include cereals (predominantly wheat, rice or maize), cereal by-products, vegetable proteins and by-products of human food production.

¹⁶ An exception to this is geese, which can live entirely on grass and similar forage.

¹⁷ Available at: <http://faostat.fao.org>

¹⁸ www.fediaf.org

Many pet food manufacturers produce standard and premium quality brands for dogs and cats. While both normally contain some cereals, levels tend to be higher in the standard brands, and may be as high as 65 % (B.M. Paragon, 2011, personal communication).¹⁹ Dog food also tends to contain more cereals than cat food (J.M. Fremy, 2011, personal communication). In the absence of any general information on ingredients used in dog and cat food in the EU, data from France¹⁸ have been used to estimate exposure of cats and dogs to Ni. These values, together with estimates of intake should be regarded as being indicative only, and will vary depending both on the availability of feed materials and on the nutrient requirements of the animals.

The amounts of food consumed by dogs and cats are influenced by many factors, including breed, size, level of activity and their reproductive state. For estimating the exposure, the CONTAM Panel applied a live weight of 4 kg and a feed intake of 60 g per day of standard pet food quality for cats (Appendix A, Section A.1.3). For dogs, a live weight of 25 kg and a feed intake of 360 g per day of standard quality were assumed (Appendix A1.4).

5.2. Water intake

Ni may be present in tap water. Levels are generally low, mean values ranging from 0.1 to 1.1 µg/L (LB-UB) were reported in the opinion on Ni in food (EFSA CONTAM Panel, 2015). The amount of water consumed by livestock varies considerably, and is influenced by factors such as the physiological state of the animal, environmental temperature, the composition of the ration and the presence of salts in the water (ARC, 1980). Estimates of water consumption by livestock (litres/day) are given in Appendix C. Table C1).

6. Exposure assessment in animals

6.1. Exposures based on concentrations in compound feeds and forage

For many livestock, feed is supplied in the form of commercially produced compound feed, and as reported in Section 4.2, data on the Ni content of 317 compound feeds were provided. However, the species for which the feeds had been manufactured or to which they were intended to be fed was not recorded; when questioned the provider of the samples reported that the samples had been collected from a range of farm types and that each sample was a mix of different feed materials used to feed cattle, pigs and poultry. As a result, it has not been possible to use these data to estimate exposure for any particular livestock category.

However, assuming these are representative of levels of Ni in compound feeds, and using the mean Ni concentrations in forages (mean LB = 650.8, UB = 651.5 µg/kg DM) for ruminants and horses, estimates of exposure have been made. Details of intake of compound feed (and forages, where appropriate) are given in Appendix A1.

In Tables 6–9 below only UB values are presented since these are used in the risk characterisation. Using upper bound values is in line with a conservative approach. Moreover, the differences between LB and UB values were, in most instances, marginal.

Table 6: The estimated mean upper-bound chronic exposure to nickel (Ni) of livestock based on mean concentrations in compound feeds and forage

Livestock category	Dietary concentration of Ni (µg/kg DM)	Total intake µg per day	Total Ni intake µg/kg b.w. per day
Dairy cows: high yielding	430	8 903	13.7
Beef: intensive cereal	884	8 836	22.1
Beef: fattening	212	2 034	5.09
Sheep: lactating	630	1 765	29.4

¹⁹ Based on statistics of 2010 of French association of pet food manufacturers (FACCO), <http://www.facco.fr/>.

Livestock category	Dietary concentration of Ni (µg/kg DM)	Total intake µg per day	Total Ni intake µg/kg b.w. per day
Goats: lactating	819	2 784	46.4
Goats: fattening	672	1 008	25.2
Pig starter	1 028	1 028	51.4
Pig finisher	1 028	3 084	30.8
Lactating sow	1 028	6 168	30.8
Chickens for fattening	1 028	123	61.7
Laying hens	1 028	123	61.7
Turkeys for fattening	1 028	411	34.3
Ducks for fattening	1 028	144	48.0
Horses	550	4 952	11.0
Salmonids	1 028	41.1	20.6
Cats	1 028	61.7	15.4
Dogs	1 028	370	14.8
Rabbits	1 028	154	77.1

b.w.: body weight; DM: dry matter.

6.2. Exposures derived from concentrations in feed materials

Where suitable data are available, the approach used above (Section 6.1) would be the most appropriate for estimating exposure to Ni by the different classes of livestock. However, due to the lack of data on the Ni content in compound feeds for individual species, a single value for all compound feeds for all species had to be used, which clearly represents a source of uncertainty. An alternative approach to estimating exposure has therefore been explored, in which example diets for a range of farm livestock and companion animals have been used, and to which Ni concentrations for individual feed materials (Table 5) have been applied.

Details of the diets are given in Appendix A. In adopting this approach, it must be stressed that the diet compositions used are not ‘average’ diets. Rather, they are intended to provide an indication of likely exposure to Ni across a range of feeding systems in Europe for the different categories of livestock.

Vegetable oils are an important ingredient in diets of many livestock and companion animals, principally as a source of energy but also because of their effect on the fatty acid composition of animal products. For ruminants in particular, hydrogenated vegetable oils are preferred because they do not adversely affect rumen fermentation. As described above (Section 4.3), Ni is used as a catalyst in the hydrogenation process (Jovanovic et al., 1998) and as a result, it may be present in the feed resulting from this process. According to members of the European Oleochemicals and Allied Products Group (APAG)²⁰ levels of Ni in hydrogenated fatty acids of up to 0.02 % (0.2 g/kg) may be present depending on the original raw material being processed and the processes used.

Therefore, the CONTAM Panel have made a ‘worst-case’ exposure assessment, based on the maximum concentration assumed from good manufacturing practice of Ni in these feed materials (50 mg/kg²¹), and a maximum inclusion rate of 5 % hydrogenated oils/fats in the compound feed. The Ni derived from hydrogenated vegetable oils has been added to that originating from other feed materials in the exposure assessment below. It should be noted, however, that in practice hydrogenated vegetable oils are not widely used in diets for pigs, poultry and horses.

6.2.1. Ruminants and horses

Table 7 provides the mean UB estimated exposures to Ni by ruminants and horses.

²⁰ According to Commission Regulation (EU) No 68/2013.

²¹ According to Commission Regulation (EU) No 68/2013.

Table 7: Estimated mean upper bound dietary concentrations and chronic exposure of ruminants and horses to nickel (Ni) from diets with and without the addition of hydrogenated vegetable oil

Livestock category	With hydrogenated vegetable oil			Without hydrogenated vegetable oil
	Dietary concentration, $\mu\text{g/kg DM}$	Ni intake $\mu\text{g/day}$	Ni intake $\mu\text{g/kg b.w. per day}$	Ni intake $\mu\text{g/kg b.w. per day}$
Dairy: high yielding	1 568	32 461	50	18
Beef: intensive cereal	2 420	24 205	60	7.4
Beef: fattening	963	9 243	23	14
Sheep: lactating	1 795	5 027	84	25
Goats: lactating	2 730	9 283	155	48
Goats: fattening	1 780	2 670	66	29
Horses	1 810	16 293	36	11

b.w.: body weight; DM: dry matter.

6.2.2. Pigs, poultry and rabbits

Based on feed intake data described in Section 5 and Appendix A, and the mean LB and UB concentrations for Ni in feed materials, estimates of the exposures to Ni in diets and exposure of pigs, poultry and rabbits are given in Table 8.

Table 8: Estimated mean upper bound (UB) dietary concentrations and chronic exposure of pigs, poultry and rabbits to nickel (Ni) from diets with and without the addition of hydrogenated vegetable oil

Livestock category	With hydrogenated vegetable oil			Without hydrogenated vegetable oil
	Dietary Ni concentration, $\mu\text{g/kg DM}$	Ni Intake $\mu\text{g/day}$	Ni intake $\mu\text{g/kg b.w. per day}$	Ni intake $\mu\text{g/kg b.w. per day}$
Pig starter	3 603	3 603	180	55
Pig finisher	3 190	9 570	96	21
Lactating sow	3 345	20 072	100	25
Chickens for fattening	3 392	407	203	53
Laying hens	3 665	440	220	70
Turkeys for fattening	3 374	1 350	112	29
Ducks for fattening	3 857	540	180	63
Rabbits	3 062	459	230	42

b.w.: body weight; UB: upper bound.

6.2.3. Cats and dogs

Based on feed intake data described in Section 5 and Appendix A, and the mean LB and UB concentrations for Ni in feeding stuffs, estimates of the exposures to Ni in diets and chronic exposure of cats and dogs are given in Table 9.

Table 9: Estimated mean upper bound dietary concentrations and chronic exposure of cats and dogs to nickel (Ni) from diets with and without the addition of hydrogenated vegetable oil

Livestock category	With hydrogenated vegetable oil			Without hydrogenated vegetable oil
	Dietary Ni concentration, $\mu\text{g/kg DM}$	Ni Intake $\mu\text{g/day}$	Ni intake $\mu\text{g/kg b.w. per day}$	Ni intake $\mu\text{g/kg b.w. per day}$
Cats	2 651	159	40	2.3
Dogs	2 651	954	38	2.2

b.w.: body weight; DM: dry matter.

6.2.4. Estimation of nickel intake by fish

Although fish are exposed to both dietary and waterborne Ni, only exposure to Ni from feed is presented in this Opinion. Based on the feed consumption presented in Section 5.1.5 and the calculated mean UB levels of Ni the estimated mean UB dietary concentration is 532 µg/kg DM. This figure is towards the lower end of the range for fish feeds and represents an estimated exposure to Ni by a 2 kg salmon of 21 µg/day. Expressed on a body weight basis this is equivalent to 10.6 µg/kg b.w. per day, respectively.

6.3. Exposure to nickel from water

As discussed elsewhere in this Opinion, Ni may be present in drinking water and in the atmosphere, but generally at very low levels, unlikely adding substantially to the overall exposure. For example, the Agricultural Research Council (ARC, 1980) suggested that the total water intake of a lactating dairy cow (600 kg b.w., 30 kg/day milk production, environmental temperature 21–25 °C) is 133 kg/day. Assuming a mean UB concentration of Ni in tap water of 1.0 µg/L (EFSA CONTAM Panel, 2015), exposure from this source would be 146 µg/day, equivalent to 0.22 µg/kg b.w., which compares with an estimated exposure from feed of 50 µg/kg b.w. (Table 7). Estimates for other livestock and companion animals (see Appendix C for details) suggest that water is likely to account for less than 1 % of total exposure.

6.4. Exposure from other sources

Nickel is naturally present in soil, and for grazing animals soil ingestion may be a major route for Ni intake. Using the titanium content of faeces as an indicator of soil ingestion, Thornton and Abrahams (1983) found that grazing cattle involuntarily ingest from 1 % to nearly 18 % of their DM intake as soil; sheep may ingest up to 30 %. However, soil ingestion varies seasonally and with farm management practices. It might be assumed that non-ruminants (e.g. free-range pigs and poultry) also consume soil during the course of their foraging, but no data have been identified to quantify this.

Ni concentrations in soils vary widely. The UK Soil and Herbage Survey reported that total concentrations in rural soils ranged from 1.16 to 216 mg/kg, with a mean value of 21.1 mg/kg (Environment Agency, 2007). Based on 20.7 kg DM intake per day for dairy cows, this would imply a daily soil ingestion of up to 3.7 kg, and 78 mg Ni (equivalent to 120 µg/kg b.w.). For mature lactating sheep (60 kg b.w.) with a feed DM intake of 2.8 kg DM, this would equate to intakes of Ni of 17.7 mg/day (295 µg/kg b.w.). Therefore, under conditions of high soil intake or high contamination levels, soil may represent a substantial contribution to Ni exposure by grazing livestock.

Sewage sludge is widely used as agricultural manure (Section 1.4), and may be applied to land on which livestock graze. Since the sludge may contain appreciable levels of Ni, this clearly represents a potential additional source of exposure. Reference has already been made to the potential uptake of Ni from the soil as a result of sludge application, but grazing livestock are also potentially at risk from sludge physically adhering to the surface of the leaves. In order to minimise this risk, Council Directive 86/278/EEC,²² which specifies controls on the use of sewage sludge in agriculture, prohibits the application of sludge onto grassland to be grazed or forage crops to be harvested within a minimum of three weeks of grazing or harvesting.²³ Clearly, the amount of sewage sludge that adheres to grassland or forage crops will be influenced by both the amount applied and subsequent rainfall before grazing commences.

Section 4.3 described the potential migration of Ni from food or feed manufacturing equipment. Machinery used in the manufacture of livestock feeds is frequently made of stainless steel, and it is possible that traces of Ni may occur in feed as a result of processing using this equipment. However, the CONTAM Panel has been unable to identify any data to quantify this.

²² See footnote 7 on Council Directive 86/278/EEC.

²³ This period may be extended by individual Member States taking account of their particular geographic and climatic conditions, but it may not be less than three weeks.

7. Hazard identification and characterisation

7.1. Toxicokinetics

7.1.1. Humans

Several reviews provide information on the toxicokinetics of Ni in humans (US EPA, 1986; WHO, 2000; ATSDR, 2005; EFSA, 2005; EU RAR, 2008; EFSA CONTAM Panel, 2015). Based on the most recent evaluation (EFSA CONTAM Panel, 2015), this section shortly summarises human toxicokinetics of Ni following oral uptake.

In humans, the bioavailability of ingested Ni ranges from 1 % up to 40 %. In particular, absorption is lower when exposure occurred in the presence of food or under non-fasted state, than when Ni was dosed in drinking water in the absence of food, or under fasted state.

After absorption Ni can bind to serum proteins and is widely distributed in the organism.

Ni is actively transferred across the blood-placental barrier into the foetus. The foetus might be particularly sensitive towards the adverse effects of Ni because it lacks effective means for eliminating excessive Ni.

Absorbed Ni is excreted mainly via the urine and, to a lower extent in breast milk. An estimated elimination half-life of 28 ± 9 hours was calculated in human volunteers.

7.1.2. Laboratory animals

As for humans, previous reviews provide information on the toxicokinetics of Ni in laboratory animals (US EPA, 1986; WHO, 2000; ATSDR, 2005; EFSA, 2005; EU RAR, 2008; EFSA CONTAM Panel, 2015). Based on these reviews the toxicokinetics of Ni following oral uptake of soluble Ni compounds in laboratory animals may be summarised as follows:

In laboratory animals Ni is rapidly but poorly absorbed following ingestion, as suggested by the low urinary excretion observed in different studies.

Upon dosing with various soluble Ni compounds, Ni was found predominantly in the kidneys. Substantial levels of Ni were also found in the liver, heart, lung, and fat as well as in the peripheral nerve tissues and in the brain.

In mice and rats, exposed to Ni during gestation, Ni was shown to cross the placenta, resulting in increased levels in the foetuses.

7.1.3. Livestock

In this section two types of data relevant for Ni toxicokinetics in livestock species are reviewed, namely:

- i) data derived from studies carried out administering to the animals controlled diets supplemented with known levels of specific forms of Ni (in general Ni^{+2}), or
- ii) data from investigations carried out on animals living in particular areas contaminated with high levels of Ni (in general only known as total Ni).

Several complications may be met in the interpretation of data relating to Ni as a contaminant in feed due to uncertainties regarding the chemical form of Ni and the levels present in soil and forage or other sources. The quantity of soil ingested with pasture by some animals, the mechanism of absorption of Ni in the blood and the presence or absence of other antagonistic metals that can all interact and affect the rate and extent of accumulation of Ni (Hausinger, 1993; Erdogan et al., 2002;

Youde, 2002). Both types of data are reviewed here in view of their potential usefulness in understanding the modalities of absorption, distribution, metabolism and excretion of the ingested Ni. The present section does not consider results of investigations, carried out with rather low levels of Ni dietary supplementation (often in the order of $\mu\text{g/kg}$ rather than mg/kg feed) and mainly intended to investigate beneficial rather than adverse effects of Ni in different livestock species (Spears, 1984; Bersényi et al., 2004; Prasad and Gowda, 2005; Schaumlöffel, 2012).

7.1.3.1. Ruminants

O'Dell et al. (1971) determined tissue distribution and excretion of Ni in groups of 3 male Holstein calves ingesting for 8 weeks (from 13 to 21 weeks of age) a basal diet supplemented with 0, 62.5, 250 or 1 000 mg/kg elemental Ni as nickelous carbonate corresponding to average daily supplemental Ni intakes of 0, 0.4, 1.3 and 1.6 g Ni, respectively. The total amount of added Ni excreted with faeces was 97.3 %, 98.1 % and 95.8 % of the added Ni in the three treated groups, respectively, while only very low levels of Ni were excreted by the animals whose diet was not supplemented. Tissue Ni distribution at the two lower Ni levels did not differ from that in control animals. However, at the highest Ni dose tested, despite the large reduction in feed intake and rate of weight gain seen, a highly significant increase in Ni content was significantly increased in different tissues in the following order: serum > kidney > vitreous humour > lung > testis > bile > tongue > pancreas > rib²⁴ > spleen > brain. Ni levels in liver and heart did not differ statistically significantly at any dose from controls.

Samples of liver, kidney and muscle tissue of 41 calves raised for 8–12 months in an area with soil rich in metals, such as copper, Ni and chromium, were collected at slaughter together with representative samples of soil and forage from 10 farms in Spain (Miranda et al., 2009). Ten representative samples of soil or forage were randomly collected from each specific field and pooled to form composite samples of soil or forage. Concentrations of Ni were significantly higher in kidneys than in liver (mean concentrations 49.3 and 10.6 mg/kg , respectively), although 53.6 % of liver samples and 35 % of kidney samples did not contain detectable amounts of Ni. Accumulation of Ni varied from non-detectable levels to a maximum of 1 758 mg/kg in kidney (median value 42.3 mg/kg). A significant increase in Ni in kidney was associated with higher concentrations of total extractable Ni in soils (ranging from 0.10 to 41.3 mg/kg) and in forage (ranging from 11.1 to 39.3 mg/kg).

Skalická et al. (2012) investigated the occurrence of cadmium (Cd), Ni and lead (Pb) in the muscle and liver of cattle from a farm near an industrial plant in Eastern Slovakia. The maximum levels of Cd, Pb and Ni were recorded in the liver (0.865, 2.324, and 1.140 mg/kg , respectively) and muscle (0.300, 0.854, and 0.700 mg/kg , respectively).

Tabinda et al. (2013) determined concentrations of heavy metals (Cu, Cd, Pb, Ni and Cr) in water, fodder, milk, meat, blood, kidney and liver of livestock (cattle and goat, no distinction reported) from two villages in the vicinity of a polluted drain in Pakistan. The order of metal concentration detected in water and fodder collected from these two villages was Cu > Ni > Pb and Cu > Ni > Pb > Cd, respectively. In milk, meat and blood the order was Cu > Ni > Pb > Cd, but in kidney and liver it was Cu > Cr > Ni > Pb > Cd, respectively. The average concentrations of Ni in livestock ranged between 4.8 ± 4.4 (1.4–8.2) and 5.6 ± 3.9 (1.7–9.5) mg/kg in milk, 2.4 ± 2.1 (0.3–4.5) and 4.5 ± 4.3 (0.2–8.8) mg/kg in meat, 4.3 ± 2.0 (2.3–6.3) and 6.5 ± 2.2 (4.3–8.7) mg/kg in blood, 0.8 ± 0.6 (0.2–1.4) and 2.4 ± 0.4 (2–2.8) mg/kg in kidney and 3.2 ± 0.4 (2.8–3.6) and 3.2 ± 1.0 (2.2–4.2) mg/kg in liver. In this short communication the numbers of analysed samples of each tissue, detailed sample analysis as well as method validation parameters, including LODs and LOQs, are missing. The CONTAM Panel noted that due to the abovementioned limitations, the rather high Ni concentrations reported in milk and tissue should not be overvalued.

Spears et al. (1978) fed lambs with a basal purified diet low in Ni (65 $\mu\text{g/kg}$) or with the basal diet adding 5 mg/kg NiCl_2 for a 97 day period. On day 94, each lamb was administered a single oral dose of radioactive nickel chloride ($^{63}\text{NiCl}_2$) in a gelatine capsule. Total urine and faecal collections were

²⁴ The 13th rib, midway between sternal end and neck.

made for 72 hours post dosing at which time the lambs were sacrificed. Radiolabelled oral Ni doses tended to be excreted to a greater extent and retained to a lesser extent in the tissues of the low dosed Ni lambs as compared to the lambs receiving high Ni doses (addition of 5 mg Ni/kg). After 72 hours of dosing the low dosed Ni lambs had excreted 74.4 % of a total oral dose of radiolabelled Ni via the faeces, while the lambs receiving Ni supplementation had excreted only 64.7 %. However, this difference was not statistically significant. Urinary excretion of Ni was low in both groups. Lambs receiving supplemental Ni tended to retain a greater concentration of radiolabelled Ni in the kidney, lung and liver after 72 hours of dosing. The kidney showed the greatest amount of radiolabelled Ni and was the only organ to retain a substantial amount of the radioactive Ni.

In a study of Khan et al. (2013) in rams the mean forage concentrations ranging from 10.34 to 20.50 mg Ni/kg corresponded to mean Ni concentrations ranged in blood plasma ranging from 0.014 to 0.024 mg/L. Notably mean blood Ni concentration in this study was much higher than the normal value for blood plasma as established previously by Puls (1994).

7.1.3.2. Pigs

Three groups of 10 one-day old pigs were fed a low Ni (0.16 mg/kg) liquid milk-based diet supplemented with 0, 5 or 25 mg NiCl₂/kg (based on a DM basis) for 21 days. At the end of the liquid feeding period, five pigs per treatment group were sacrificed and the remaining five were fed a dried skim milk-based diet (0.12 mg/kg Ni) with similar levels of added Ni for an additional 28 days (Spears et al., 1984). At the end of the 21 day liquid feeding period, the addition of 25 mg/kg Ni to the basal diet increased liver, kidney, lung and serum Ni concentrations ($P < 0.05$), whereas at the lower level of supplementation Ni concentrations of 5 mg/kg Ni increased ($P < 0.05$) only in the kidney and lung. At the end of the dry feeding phase, tissue Ni concentrations showed a different pattern in the three groups. Animals that received 25 mg/kg Ni for the entire experimental period of 49 days had increased concentrations of Ni in kidney, spleen, muscle and serum. The low level of supplemental Ni did not significantly increase Ni concentrations in any of the tissues examined at 49 days. In the animals that were killed after 21 days, the highest concentration of Ni was found in the kidney of animals supplemented with Ni. However, at the end of the 49-day study, concentrations of Ni were similar in lung and kidney. The addition of 5 mg/kg Ni to the basal liquid diet increased liver iron concentration ($P < 0.05$) and tended to increase spleen iron concentration at 21 days. No differences in tissue iron concentrations were noted between animals consuming the 0 or 5 mg/kg Ni diets at the end of the 49-day study. However, at 49 days iron concentrations were higher in lung tissue of pigs receiving 25 mg/kg Ni compared to those supplemented with 0 or 5 mg/kg Ni.

7.1.3.3. Rabbits

Following dietary supplementation with 50 and 500 mg Ni (as NiCl₂) per kg feed to adult female rabbits, approximately 98 % of the Ni load was excreted from the body via the faeces and 0.5–1.5 % with the urine, whereas approximately 1 % was retained in the body (Bersényi et al., 2004). Absorbed Ni accumulated in the kidneys, bones, heart and liver of adult female rabbits. When rabbits ingested 6.2 ± 1.2 ; 59.3 ± 9.5 and 506.5 ± 120.4 mg/kg of Ni⁺² in the form of NiCl₂ in feed (corresponding to 0, 50 and 500 mg/kg Ni in feed), the Ni content of the organs investigated was significantly increased with the Ni load. Ni accumulated in the kidneys, bone, heart, liver and lungs. The ovaries had relatively high concentrations of Ni, i.e. 3–4 times higher than in control animals. With increasing the Ni-load, the Ni content of muscle was significantly increased (0.02 ± 0.01 ; 0.07 ± 0.01 and 0.15 ± 0.02 mg/kg DM, corresponding to 0, 50 and 500 mg/kg Ni in feed, respectively). Ni load of the body did not affect the Cu, Zn, Fe, and Mn concentrations of the different organs in rabbits, with the exception of the liver, spleen and ribs where significantly ($P < 0.05$) reduced concentrations of Cu were observed.

No accumulation of Ni and iron was observed in testes and epididymis of rabbits fed for 90 days a granular mixture with addition of 175 or 350 mg Ni/kg feed (Kalafová et al., 2012a).

Kalafová et al. (2012b) investigated the effect of dietary Ni and a combination of Ni and Zn on the accumulation of Pb, Cd, Ni and Zn in muscles, liver and kidneys of adult female rabbits. The inclusion of Ni (175 or 350 mg NiCl₂/kg) and Zn to the diet for rabbits for 90 days had no effect on the concentration of Ni and Zn in liver, kidney and muscle (*musculus biceps femoris* and *musculus longissimus dorsi*). The addition of Ni caused an increase in Cd concentration in rabbit kidneys. In the liver, addition of Ni resulted in an insignificant decrease of Cd concentration but Zn addition at the amount of 300 mg/kg to the diet caused an increase of Cd levels in the kidney as well as in the liver. Ni and Zn treatment caused a decrease of Pb accumulation in the *musculus longissimus dorsi* of rabbits.

7.1.3.4. Poultry

In the study by Rehman et al. (2012) on translocation of zinc and Ni from poultry feed to broilers and their excretion through litters, the maximum contents of Ni, corresponding to Ni levels of between 26 and 33 g/kg broiler feed, were found in muscles (125 ± 1.0 mg/kg), while liver accumulated lower concentrations of Ni (101 ± 0.90 mg/kg) that were, however, higher than in skin (66 ± 0.48 mg/kg).

Wilson et al. (2001) administered 0, 25, 50, 75, 100 and 150 mg/kg dietary Ni given as NiCl₂ chloride to groups of 50 one-day broiler chicks for six weeks. The Ni concentrations in the bone tissue of the broilers supplemented were 0.07, 0.07, 0.05, 0.09, 0.18 and 0.34 mg/kg, respectively. Only the group receiving the highest dose of 150 mg/kg had statistically significantly higher concentrations than the control group. Ni concentrations in bone were similar to those reported by Ling and Leach (1979) who determined Ni concentrations in 3-week-old male broilers fed diets supplemented with 0, 300 and 500 mg/kg Ni and found Ni concentrations in the bone of 0.1, 0.97, and 1.88 mg/kg, respectively.

Thirty breeding pairs of mallard ducks were randomly assigned to one of five treatment groups and were fed breeder mash containing 0, 12.5, 50, 200 or 800 mg/kg Ni (given as NiSO₄) for 90 days (Eastin and O'Shea, 1981). Although resulting absolute concentrations of Ni in different tissues were low, there was a significant accumulation, compared to controls, in the kidneys of birds fed Ni at all concentrations, and in feathers, blood, and livers of birds fed the two highest concentrations of Ni.

7.1.3.5. Fish

An 18-day experiment was conducted by Ptashynski et al. (2001) to investigate the uptake of dietary Ni administered in the form of NiSO₄ in adult lake whitefish (LWF) and lake trout (LT) fed diets containing 0, 1000 and 10 000 mg Ni/kg, prepared with and without brine shrimp. Increased Ni concentrations in all LWF tissues, except the intestine, were associated with increased doses of Ni. The LWF had elevated concentrations of Ni in kidney and liver, at mean concentrations of 4.7 and 0.57 mg Ni/kg, respectively. A similar range of concentrations was observed in LWF fed 1 000 mg Ni/kg (without brine shrimp) and 10 000 mg Ni/kg (without brine shrimp), with concentrations of 4.0 and 5.1 mg Ni/kg in kidney and concentrations of 0.49 and 0.63 mg Ni/kg in liver, respectively. Conversely, the concentrations observed in kidney and liver of LT fed low dose diets were much higher, with mean concentrations in the kidney and liver ranging from 6.4–7.6 to 1.1–1.2 mg Ni/kg, respectively.

The accumulation and distribution of Ni in natural populations of freshwater fish residing in Ni-contaminated habitats have been investigated in several field studies (Kashulin and Reshetnikov, 1995; Moiseenko et al., 1995; Allen-Gil et al., 1997; Klaverkamp et al., 2000). Metal accumulations in fish from natural populations in contaminated habitats are due to uptake from both water and ingestion of food and sediments (Handy, 1996). Whitefish collected from the contaminated lake, located near base-metal mining smelters in Russia, accumulated 5.6 mg Ni/kg (wet weight) in kidney, 1.92 mg Ni/kg (wet weight) in gill, and 1.5, 0.5 and 0.3 mg Ni/kg (wet weight) in skeleton, liver and muscle, respectively (Kashulin and Reshetnikov, 1995).

In a study from Klaverkamp et al. (2002) lake whitefish (LW) collected from the Little Macdonald Lake in Key Lake, Saskatchewan (with sediment Ni concentrations as high as 690 mg Ni/kg (dry

weight), from mining activity) had elevated Ni levels in kidney and liver. It appears, therefore that accumulation of Ni in dietary exposed lake trout in the Ptashinsky et al. (2001) study takes place in different concentrations and patterns than those observed in natural populations of freshwater fish collected from contaminated habitats (Dallinger and Kautzky, 1985; Bradley and Morris, 1986; Kashulin and Reshetnikov, 1995; Allen-Gil et al., 1997). For example, LT collected from Lake Nelson, located near mining operations in Sudbury, Ontario, accumulated mean concentrations of 1.0 mg Ni/kg (wet weight) in kidney and < 0.4 mg Ni/kg (wet weight) in liver (Bradley and Morris, 1986). Concentrations of Ni in the superficial sediments of Lake Nelson were 444 mg Ni/kg (dry weight).

Adult rainbow trout were pre-exposed to a concentration of waterborne Ni (7.43 µmol/L) or a control water (0.12 µmol/L) for 45 days, and subsequently, a gastrointestinal dose of radiolabeled Ni (1.08 µmol/L wet weight) was infused into the stomach of both non-pre-exposed and Ni pre-exposed trout to test whether pre-exposure to waterborne Ni would affect gastrointestinal uptake (Chowdhury et al., 2008). The fish pre-exposed to waterborne Ni exhibited a markedly greater level of total Ni in the blood plasma (approximately 10-fold) compared to those not pre-exposed but not in red blood cells (RBC). Pre-exposure down regulated the gastrointestinal uptake of the radiolabeled Ni (new Ni) in the plasma and RBCs, providing evidence of homeostatic interaction between the two routes of Ni uptake. The plasma and RBC concentrations radiolabeled Ni in the non-pre-exposed and Ni pre-exposed groups were linear in the first two hours and then approached a plateau. Only a small fraction of the infused dose (1.6–3.7 %) was found in the internal organs of both groups at 24 hours. Waterborne Ni, but not the infused Ni, greatly increased total Ni levels in the gills (6.1 fold), kidney (5.6 fold), scales (4.2 fold), and gut tissues (1.5–4.2 fold). It appears that gut, kidney and scales play important roles for Ni homeostasis by providing uptake, clearance and storage sites.

7.1.3.6. Horses

Although the CONTAM Panel has not identified any studies on the toxicokinetics of Ni in horses, levels of Ni observed in the mane hairs in a study by indicate bioavailability of Ni in this species.

7.1.3.7. Dogs

The CONTAM Panel has not identified any studies on toxicokinetics of Ni in dogs. However, Ni levels measured in serum indicate bioavailability of Ni in this species (Mert et al., 2008; Tomza-Marciniak et al., 2012), however, cannot be quantified from the available information.

7.1.3.8. Cats

The CONTAM Panel has not identified any studies on toxicokinetics of Ni in cats.

7.1.4. Carry-over of nickel in feed to animal derived food

7.1.4.1. Carry over from nickel in feed to milk

In 1949, Archibald undertook a study with lactating dairy cows fed diets, the daily ration of which were supplemented with 500 mg Ni hexahydrate chloride (equivalent to 145 mg elemental Ni per day)

for two months. Although Ni was detected in numerous samples of milk collected, it was shown that when milk was collected directly into glass jars, Ni was not present. Archibald (1949) concluded therefore that where Ni had been reported in previous studies, this was the result of contamination from the milking machine, and that Ni is not a constituent of natural milk under the tested conditions. The CONTAM Panel noted that in this study a spectrophotometric method was used for analysis the sensitivity of which may not have been sufficient to accurately quantify levels of Ni in milk.

Kirchgessner et al. (1967) reported that bovine milk normally contains about 0.2–0.5 mg Ni/L, and that the concentration is not influenced by dietary Ni intake. O'Dell et al (1970a) reported that supplementation of dairy cow diets with 365 or 1835 mg Ni/day did not result in an increase in the Ni

content of milk. Kirchgessner et al. (1967) however, reported a four-fold increase in the Ni content of colostrum compared to milk.

The CONTAM Panel noted that these studies do not allow any estimation of carry over rate to be determined. Data presented in Table 15 (Section 9) give levels of Ni in milk although it is not clear whether the origin was feed or contamination during processing. Leeman et al. (2007) estimated a transfer factor in whole milk of only 0.024. The respective transfer factor is expressed as the concentration of Ni in whole milk divided by its concentration in animal feed, in which the concentration in animal products is on a wet weight basis, and in feed on a dry weight basis.

7.1.4.2. Carry-over of nickel in feed to animal tissues

Leeman et al. (2007) carried out a meta-analysis, covering references from the literature as well as confidential data regarding the transfer of various chemicals from feed to animal products. They calculated transfer factors, which are expressed as the concentration of the respective compound in the animal product (mg/kg wet weight) divided by the concentration of the compound in animal feed (mg/kg dry weight). For Ni they present the following transfer factors (95th percentile): meat 0.58, fat 0.12, liver and kidney 0.70.

In Table D1 of Appendix D an overview of important Ni transfer studies is presented. The data provided clearly show that carry over of Ni occurs to some extent but it has not been possible to calculate a carry-over rate.

7.1.5. Conclusions on toxicokinetics

Most of the available data obtained in livestock animals following administration of Ni deal with Ni²⁺ applied as Ni chloride or sulphate. In general, livestock animals absorb only a small proportion of the total oral Ni intake. The extent of gastrointestinal absorption of Ni depends on several factors, which are:

- (i) the animal species considered;
- (ii) the amount of administered Ni;
- (iii) its chemical form;
- (iv) interactions with other elements, and
- (v) the vehicle used for Ni administration (e.g. solid diet, liquid diet or water).

A very large proportion of the ingested Ni remains unabsorbed and is excreted in the faeces, while the small fraction of absorbed Ni is excreted primarily via the urine. ATSDR (2005) reported that faecal Ni excretion is usually about 100 times that of urinary Ni excretion.

Absorbed Ni binds to specific proteins and/or amino acids in the blood serum and is rapidly transported and distributed to different organs and tissues. In ruminants following Ni ingestion Ni concentrations increase considerably in kidney and other organs and tissues such as lung, liver, muscles, testis, pancreas and spleen. Absorbed Ni is also partly excreted via milk. Data available from rabbits and poultry show that, Ni also accumulates in bones. In female rabbits, increased Ni concentrations were also observed in the ovaries. In fish, Ni accumulates mainly in kidney, gill, skeleton, liver and muscle, in conjunction with increased metallothionein concentrations in kidney and liver. In rainbow trout waterborne Ni accumulated in the gill, while direct infusion of Ni into the bloodstream leads to extrabranchial accumulation of Ni only in the kidney.

The data provided show that carry-over of Ni occurs, but it has not been possible to calculate a carry-over rate from feed to animal tissue.

7.2. Toxicity in experimental animals and humans

An extensive and comprehensive evaluation of Ni toxicity in experimental animals and humans is presented in the opinion on Ni in food (EFSA CONTAM Panel, 2015).

Briefly, based on the overall evidence evaluated in the opinion on Ni in food, developmental toxicity was identified as the critical effect for the assessment of chronic effects of Ni. A tolerable daily intake (TDI) of 2.8 µg Ni/kg b.w. per day was derived from the lowest BMDL₁₀ (lower 95 % confidence limit for a benchmark response at 10 % extra risk) of 0.28 mg/kg b.w. for post-implantation foetal loss in rats. The systemic contact dermatitis (SCD) elicited in Ni-sensitive humans after oral exposure to Ni was identified as the critical effect for the assessment of acute effects of Ni. A lowest BMDL₁₀ of 1.1 µg Ni/kg b.w. was derived for the incidence of SCD following oral exposure to Ni of human volunteers. The CONTAM Panel applied a margin of exposure (MOE) approach and considered an MOE of 10 to be indicative of a low health concern.

7.3. Adverse effects of nickel in livestock, fish, cats and dogs

The present Section does not deal with studies, carried out with relatively low levels of Ni (i.e. in the order of µg/kg rather than mg/kg feed) given as dietary supplements and designed to investigate beneficial effects of Ni (Spears, 1984; Bersényi et al., 2004; Prasad and Gowda, 2005; Schaumlöffel, 2012).

Dietary Ni requirement for monogastric species appear to be between 0.050 and 0.200 mg Ni/kg of feed (Samal and Mishra, 2011). Signs of Ni deprivation have been described for several livestock species including chickens, cows, goats, minipigs and sheep. Since normal feeding situations usually provide Ni in excess of this amount (see Section 6), a primary deficiency of Ni is unlikely to occur under practical conditions.

Studies in which Ni was administered intraperitoneally or via inhalation are also not considered in the present opinion because these exposure routes are not appropriate for dietary hazard characterisation.

For derivation of NOAELs and LOAELs in mg Ni/kg b.w. per day from Ni/kg feed levels given in the studies below feed intake values and body weights for the different animal species as presented in Appendix A have been used.

7.3.1. Adverse effects in ruminants and monogastric livestock animals

7.3.1.1. Cattle

Ni appears to be relatively non-toxic for ruminants (O'Dell et al., 1970b) and adverse effects appear to reflect the susceptibility of the rumen microflora towards Ni rather than that of the host animal. Calves (105 kg) were fed diets containing 62.5, 250 and 1 000 mg Ni/kg (given as nickel carbonate: NiCO₃) for 8 weeks. Animals receiving 250 mg Ni/kg feed showed slightly reduced feed intake and growth rates as compared to controls. These same effects were more pronounced in the high dose group. These adverse effects appeared to be associated with a reduction in feed intake as a result in rumen function and reductions in nitrogen retention and organ size. When animals previously fed with Ni added diets were reversed to a diet not containing added Ni, no effects on growth or feed intake were observed. The CONTAM Panel identified a NOAEL of 1.34 mg Ni/kg b.w. per day corresponding to the lowest dose (62.5 mg Ni/kg feed) in this study.

The CONTAM Panel did not identify relevant studies on sheep or goats.

7.3.1.2. Pigs

NRC (2005) reported that no adverse effects were observed in pigs fed diets up to 400 mg/kg Ni (given as sulphate or acetate).

Kirchgessner and Roth (1977) applied Ni (as sulphate) at concentrations of 125, 250, 375 and 500 mg Ni/kg feed with pig starter and rearing diets to young pigs for 6 weeks. Slight reductions in weight gain and feed intake were observed at 375 mg Ni/kg feed and above. The effects observed were more pronounced at the highest concentration. The CONTAM Panel derived a NOAEL of 250 mg/kg feed corresponding to 12.8 mg Ni/kg b.w. per day from this study for pigs.

Spears et al. (1984) fed a low Ni (0.16 mg/kg) liquid milk-based diet supplemented with 0, 5 or 25 mg Ni/kg (given as NiCl₂) to three groups of 10 one day-old pigs for 21 days. At the end of the liquid feeding period, five pigs per treatment group were sacrificed. The remaining animals were fed a dried skim milk-based diet (0.12 mg Ni/kg) supplemented with similar levels as before for additional 28 days. Pigs receiving the highest concentration of Ni (25 mg Ni/kg feed) during the full study period showed decreased serum alkaline phosphates and increased serum glucose. No effects on weight gain, liver cholesterol, serum protein concentrations or bacterial urease activity in the gastrointestinal tract were observed. In the absence of other findings, the CONTAM Panel considered the blood chemistry changes as not adverse.

7.3.1.3. Rabbits

Adult female rabbits were exposed to 0, 50 or 500 mg Ni/kg feed (given as NiCl₂) for five consecutive weeks (Bersényi et al., 2004). No significant changes were observed in body weight gain in exposed animals in comparison to controls. A statistically significant decrease in relative weights of liver, kidneys and ovaries was observed in animals exposed to 500 mg Ni/kg. The study authors reported that 'histopathological investigations indicated that the activity of ovaries was reduced' in the 500 mg Ni/kg group, without providing details on the nature of changes observed. No significant differences were observed in the digestibility of crude proteins and fibre in the groups exposed to Ni in comparison to controls. The CONTAM Panel derived a NOAEL of 3.75 mg/kg b.w. per day for rabbits (corresponding to 50 mg Ni/kg feed) for this study.

Kalafovà et al. (2008) investigated the effects of administration of Ni alone and Ni together with zinc on growth, total protein and cholesterol concentration in female rabbits in a 90 day study. Animals exposed to only Ni received 17.5 or 35 mg NiCl₂/kg in feed whereas the groups exposed to Ni and zinc were exposed to concentrations of 17.5 mg NiCl₂/kg + 30 mg ZnCl₂/kg or 35 mg NiCl₂/kg + 30 mg ZnCl₂/kg. No effect of Ni or zinc on growth, and total protein and cholesterol concentration was observed.

Martiniaková et al. (2009) studied the effects of dietary supplementation of Ni alone and Ni together with zinc on femoral bone structure in a 90 day study with young rabbits. Fifteen one month-old female rabbits were divided into three groups of five animals each. One group received feed containing 350 mg NiCl₂/kg the second group received feed containing 350 mg NiCl₂ and 300 mg ZnCl₂/kg and the last group served as control. Ni alone affected size of the osteons. The combination of Ni and Zn affected in addition also the microstructure of compact bone tissues.

Table 10 presents an overview about the available relevant toxicity studies with Ni in cattle, pigs and rabbits.

Table 10: Relevant nickel (Ni) toxicity studies with cattle, pigs and rabbits

Species Doses Exposure duration	NOAELs	Ni levels associated with adverse effects	Adverse effects observed	References
Cattle (calves); 62.5, 250 and 1 000 mg Ni/kg basal diet (given as NiCO ₃);	62.5 mg Ni/kg basal diet corresponding to 1.34 mg Ni/kg b.w. per day (based on a feed intake of 0.021 kg feed/kg b.w. per day)	250 and 1 000 mg Ni/kg basal diet corresponding to 5.34 and 21.3 mg Ni/kg b.w. per day, respectively (based on a feed intake of	Slightly reduced feed intake and growth rate	O'Dell et al. (1970b)

Species Doses Exposure duration	NOAELs	Ni levels associated with adverse effects	Adverse effects observed	References
8 weeks		0.021 kg feed/ kg b.w. per day)		
Pigs (piglets) 125, 250, 375 and 500 mg Ni/kg feed (given as NiSO ₄); 6 weeks	250 mg Ni/kg starter and rearing diet corresponding to 12.8 mg Ni/kg b.w. per day (based on a feed intake of 0.05 kg feed/ kg b.w. per day)	375 mg Ni/kg starter and rearing diet corresponding to 18.6 mg of Ni/kg b.w. and day (based on a feed intake of 0.005 kg feed/ kg b.w. per day)	Slightly reduced feed intake and body weight gain	Kirchgessner and Roth (1977)
Pigs (piglets, one day old and 1.2 kg b.w.) 5 and 25 mg Ni/kg DM per day (given as NiCl ₂); 7 weeks	25 mg Ni/kg liquid milk-based diet corresponding to 1.23 mg Ni/kg b.w. per day (based on a feed intake of 0.005 kg feed/kg b.w. per day)			Spears et al. (1984)
Rabbits (female adult) (average 4.5 kg b.w.); 50 and 500 mg Ni/ kg feed (given as NiCl ₂); 5 weeks	50 mg Ni/kg feed corresponding to 3.75 mg Ni/kg b.w. per day (based on a feed intake of 0.075 kg feed/kg b.w. per day)	500 mg Ni/ kg feed corresponding to 37.8 mg Ni/kg b.w. and day (based on a feed intake of 0.075 kg feed/ kg b.w. per day)	Reduced relative weights of liver, kidneys and ovaries and reduced function of the ovaries. Alterations of blood parameters.	Bersényi et al. (2004)
Rabbits (female) 7.9 and 15.8 mg Ni/kg feed (given as NiCl ₂); 12.9 weeks	15.8 mg Ni/kg feed corresponding to 1.18 mg Ni/kg b.w. per day (based on a feed intake of 0.075 kg feed/kg b.w. per day)			Kalafovà et al. (2008)
Rabbits (female) (average 3.8 kg b.w.); 158 mg Ni/kg feed (given as NiCl ₂); 12.9 weeks	< 13 mg Ni/kg b.w. per day	158 mg Ni/kg feed corresponding to 13 mg Ni/kg b.w. per day (based on a feed intake of 0.075 kg feed/ kg b.w. per day)	Affected microstructure of compact bone tissue	Martiniaková et al. (2009)

b.w.: body weight; DM: dry matter; NOAEL: no observed adverse effect level.

7.3.2. Poultry

Martinez and Diaz (1996) studied the effects of Ni on blood haemoglobin content and pulmonary hypertension in broiler chick. One day-old male broiler chicks were given feed containing 123, 247 and 494 mg Ni/kg (given as NiCl₂) for 6 weeks. Animals had *ad libitum* access to feed during the study period. Concentrations of ≥ 247 mg Ni/kg resulted in increased blood haemoglobin content, pulmonary hypertension and increased incidences of ascites and right ventricular hypertrophy.

Concentrations of 0, 100, 300, 500, 700, 900, 1 100 and 1 300 mg Ni sulphate or Ni acetate/kg in basal diet were administered *ad libitum* to broiler chicks for 4 weeks. Concentrations of ≥ 500 mg Ni /kg in feed and above caused reduced growth (Weber and Reid, 1968).

Ling and Leach (1979) studied Ni toxicity in male chicks receiving a purified basal diet supplemented with 0, 300, 500, 700, 900 and 1 100 mg Ni/kg given as NiCl₂ *ad libitum*. Dietary Ni concentrations of

300 mg/kg and higher caused a significant reduction in growth rates. Anaemia and mortality were observed at the highest tested Ni concentration of 1 100 mg Ni/kg.

Bersényi et al. (2004) performed a broiler chicken study to examine the effect of Ni (given as NiCl_2) supplementation on growth performance. Broilers were fed a corn-soybean based grower diet supplemented with 0, 50 or 500 mg Ni/kg DM between 14 and 49 days of age. Ni supplementation did not alter mortality, but at 500 mg Ni/kg significantly reduced body weight gain as compared to controls. Additionally, the relative weight of the liver and testicles but not of heart and spleen were significantly decreased by Ni supplementation at 50 and 500 mg/kg. Activities of serum gamma-glutamyltransferase, aspartate aminotransferase, choline esterase, alkaline phosphatase as well as the concentrations of cholesterol, triglycerides and creatinine were not significantly altered by supplementation with 500 mg Ni/kg. Histopathological analysis showed the presence of mild to moderate focal fatty infiltration in the liver of animals exposed to 50 and 500 mg Ni/kg (no additional details reported). The CONTAM Panel identified a LOAEL of 3.0 mg/kg b.w. per day (corresponding to 50 mg Ni/kg in feed) for this study.

A study with hens was carried out by Arpasova et al. (2007) in which 0, 0.02, 0.20 and 2.0 mg NiCl_2/L were administered *per os* in water for 28 days. The body weight in animals receiving 2.0 mg NiCl_2 was significantly decreased. Dose dependent reduction of egg production was seen in all groups. Albumen weight and content were significantly decreased in the highest dose group as compared to controls. It is notable that the adverse effect of Ni on egg production has been seen at unusually low levels rather in the range of Ni doses considered to be beneficial to several livestock species (Samal and Mishra, 2011). Moreover, the authors reported that body weight was significantly reduced at values of 0.20 mg/kg feed thereby contradicting the results of several other poultry studies reporting Ni induced body weight reduction only at much higher values (see Table 11). Based on the uncertainties incurred with the results of this study and further inconsistencies in their reporting, the study was not considered for risk assessment.

Young hens and cocks (24 weeks old) received feed containing 0, 250, 500 and 1 000 mg/kg Ni (given as $\text{NiSO}_4 \cdot \text{H}_2\text{O}$) *ad libitum* for 42 days (Trüpschuch et al., 1996). Fertilisation success, rate of dead, stuck (i.e. unable to break through the egg shell during hatching) and malformed chickens were assessed. Supplementation with 1000 mg Ni/kg feed exerted a significant reduction in feed intake, percentage of laying hens as well as egg weights. Moreover, the highest supplementation dose resulted in a significant number of dead, stuck and malformed chickens. Similarly to their mothers, feed of the chicken offspring was supplemented with 250, 500 and 1 000 mg Ni/kg for 28 days. Ni supplementation with 250 and 1 000 mg Ni/kg, respectively, significantly increased mortality of the chickens. The lack of effects seen in the 500 mg Ni/kg group was attributed by the authors to the small number (38) of animals used. Based on the highly significant decrease of Zn content in eggs observed in association with Ni administration, the authors concluded that the toxic effects of Ni are due to the induction of a Zn deficiency.

One-day old broilers were fed *ad libitum* a corn-soybean basal diet supplemented with 0, 300, 600 and 900 mg NiCl_2/kg for 42 days (Wu et al., 2013). The serum contents of interleukin -2, -4, -6, -10, interferon gamma and tumour necrosis factor alpha in serum and activities of superoxide dismutase, catalase and glutathione peroxidase decreased in gastrointestinal mucosa (paralleled by an increase in malondialdehyde) in gastrointestinal mucosa increased significantly in all groups as compared to controls. Since the study endpoints were considered not relevant for derivation of NOAELs/LOAELs the study was not considered for risk assessment.

Mallard ducklings were fed Ni (given in the form of sulphate) with 0, 200, 800, or 1 200 mg/kg feed from day one to day 90. Ducklings fed 1 200 mg/kg Ni showed tremor and paresis after 14 days and 71 % of them died by 60 days of age. Additionally, this dosage resulted in a decrease of body weight of the ducks at 28 days of age. Weights of ducklings in the 200 and 800 mg Ni/kg groups were not significantly different from the control group. Bone density (as assessed by the weight/length ratio of the humerus) was affected in females given 800 mg/kg Ni in the diet and for all ducklings fed

1 200 mg Ni/kg at 30 days of age (Cain and Pafford, 1981). The CONTAM Panel derived a NOAEL of 9.4 mg/kg b.w. per day (corresponding to 200 mg Ni/kg feed) based on decreased bone density at a dose of 37.6 mg/kg b.w. per day (corresponding to 200 mg Ni/kg feed).

The CONTAM Panel did not identify relevant studies on Ni toxicity in turkeys.

Table 11 presents an overview about the relevant toxicity data available for poultry.

Table 11: Relevant nickel (Ni) toxicity studies with poultry

Species Doses Exposure duration	No observed adverse effect levels (NOAELs)	Ni levels associated with adverse effects	Adverse effects observed	References
Chickens (male broiler); (from day 1) 123, 247, 494 mg Ni/kg feed (given as NiCl ₂); 6 weeks	123 mg Ni/kg feed corresponding to 7.4 mg Ni/kg b.w. per day (based on a feed intake of 0.06 kg dry weight feed/kg b.w. per day)	247 mg Ni/kg feed corresponding to 14.8 mg Ni/kg b.w. per day (based on a feed intake of 0.06 kg dry weight feed per day)	Significant b.w. reduction, increased haemoglobin and pulmonary hypertension	Martinez and Diaz (1996)
Chickens (broiler); 100–1 300 mg Ni/kg basal diet (given as Ni sulphate or acetate); 4 weeks	300 mg Ni/kg basal diet corresponding to 18 mg Ni /kg b.w. per day (based on a feed intake of 0.06 kg feed/kg b.w. per day)	500 mg Ni/kg basal diet corresponding to 30 mg Ni/kg b.w. per day (based on a feed intake of 0.06 kg feed/kg b.w. per day)	Depressed chick growth as assessed by body weight	Weber and Reid (1968)
Chickens (broiler); (from day 14 to day 49) 50 and 500 mg Ni/kg DM; 5 weeks	< 3.0 mg Ni/kg b.w. per day	50 mg Ni/kg DM corresponding to 3.0 mg Ni/kg b.w. per day (based on a feed intake of 0.06 kg feed/kg b.w. per day)	Slightly reduced relative weights of livers and testicles and mild pathological liver focal fatty infiltration together with alterations of chemical parameters	Bersényi et al. (2004)
		500 mg Ni/kg DM corresponding to 30 mg Ni/kg b.w. per day (based on a feed intake of 0.06 kg feed/kg b.w. per day)	Reduced body weight and feed conversion efficiency	
Chickens (from day 1) 300–1 100 mg Ni/kg feed (given as NiCl ₂); 3 weeks	< 18 mg Ni/kg b.w. per day	300 mg Ni/kg DM corresponding to 18 mg Ni/kg b.w. per day (based on a feed intake of 0.06 kg feed/kg b.w. per day)	Decreased growth and Ni accumulation in the kidney. Increased mortality at higher doses	Ling and Leach (1979)
Chickens (young hens and cocks ca. 24 weeks); 250, 500 and 1 000 mg Ni/kg feed (given as	< 30 mg Ni/kg b.w. per day	500 and 1 000 mg/kg feed corresponding to 30 and 60 mg Ni/kg b.w. per day	Reduced feed intake, and number of laying animals and reduced egg weight; increased percentage of dead	Trüpschuch et al. (1996)

Species Doses Exposure duration	No observed adverse effect levels (NOAELs)	Ni levels associated with adverse effects	Adverse effects observed	References
NiSO ₄ ; 42 days		(based on a feed intake of 0.06 kg/feed/kg b.w. per day)	chick embryos and of stuck and malformed chickens	
Chickens (offspring from abovementioned hens and cocks); 250, 500 and 1 000 mg Ni/kg feed (given as NiSO ₄); 28 days	< 15 mg Ni/kg b.w. per day	250, 500 and 1 000 mg Ni/kg feed corresponding to 15, 30 and 60 mg Ni/kg b.w. per day (based on a feed intake of 0.06 kg feed/kg b.w. per day)	Reduced live weight gain and feed intake at 500 and 1 000 mg. Increased mortality at 250, 500 and 1 000 mg/kg	
Ducks (Mallard ducklings); 200 –1 200 mg Ni/kg (given as NiSO ₄ sulphate); 90 days	200 mg Ni/kg of feed corresponding to 9.4 mg Ni/kg b.w. per day (based on a feed intake of 0.047 kg feed per kg b.w. per day)	800 mg Ni/kg feed corresponding to 37.6 mg Ni/kg b.w. per day (based on a feed intake of 0.047 kg feed per kg/b.w. per day)	Decreased bone density	Cain and Pafford (1981)
		1 200 mg Ni/kg feed corresponding to 56.4 mg Ni/kg b.w. per day (based on a feed intake of 0.047 kg feed per kg b.w. per day)	Tremor, paresis, decreased weight and bone density, death	

b.w.: body weight; DM: dry matter.

7.3.3. Fish

Fish may be exposed to both dietary and waterborne Ni. However, the present EFSA Opinion deals with Ni in feed and therefore only feeding studies on Ni in fish have been assessed.

Javed (2013) investigated *inter alia* the impacts of dietary exposure to Ni in juvenile Major carp (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*). Triplicate groups of fish were exposed to 70.4 mg/kg feed (*Catla catla*), 72.0 mg/kg feed (*Labeo rohita*) or 79.1 mg/kg feed (*Cirrhina mrigala*) for 12 weeks. Fish growth was monitored in terms of wet weight and fork length increments, condition factor, feed intake and feed conversion efficiency (FCE). Dietary exposure to Ni caused a significant decrease ($p < 0.05$) in weight gain (13.8 g versus 35.8 g) and fork length (15.7 mm versus 34.2 mm) compared to control fish.

Ptashynski et al. (2001) investigated the toxicity of dietary Ni exposure in triplicate groups of adult lake whitefish and lake trout fed diets containing 0, 1 000 or 10 000 mg Ni/kg for 18 days (diets were prepared with brine shrimp (analysed values: < 0.05, 1 100 or 11 000 mg Ni/kg) or without brine shrimp (analysed values 1.5, 1 500 or 14 000 mg Ni/kg)). Feed consumption ceased in both species after a few feedings in the high dose treatments. With the exception of a significant decrease of 9.8 ± 1.1 % in weight of lake trout fed 10 000 mg/kg (with shrimp), there were no significant differences in weight between groups within species. Metallothionein concentrations increased significantly in kidneys of lake trout fed 1 000 mg Ni/kg and 10 000 mg Ni/kg and lake whitefish fed 10 000 mg Ni/kg. Metallothionein levels were also significantly increased in livers of lake whitefish fed 10 000 mg Ni/kg (diet without shrimp only). Lipid peroxide production increased significantly in the plasma of lake trout fed 10 000 mg Ni/kg. Haemoglobin concentrations and haematocrit values were unaffected by dietary Ni exposure. Significant decreases in blood glucose concentrations were

observed in lake whitefish fed 1 000 mg Ni/kg and 10 000 mg Ni/kg (both with shrimp). Lake trout fed 10 000 mg Ni/kg (with shrimp) exhibited significant decreases in plasma K⁺, Cl⁻ and Na⁺ concentrations. Lake whitefish fed 10 000 mg Ni/kg (without shrimp) had K⁺ concentrations that were significantly lower than in control fish. Histopathological alterations were observed in kidneys of lake whitefish fed low and high dose Ni-containing diets, and in both livers and intestines of fish fed the high dose diets. Lake trout fed low and high dose diets exhibited similar histological alterations in intestines to those observed in lake whitefish. The most marked histological alterations in this study were observed in posterior kidneys of lake whitefish fed high dose diets, indicating that kidney may be a target organ for Ni toxicity in this species.

In a subsequent sub-chronic study, Ptashynski et al. (2002) examined the toxicity of dietary exposure of adult lake whitefish to 0, 10, 100 or 1 000 mg Ni/kg (analysed values: 1.1, 12, 110 or 1 100 mg Ni/kg) for 10, 31, and 104 days. Haematological parameters (concentrations of glucose, haemoglobin and haematocrit), organ and whole organism parameters (liver somatic index, growth, and condition factor) did not differ between control and treated fish. Histopathological lesions in kidney and liver were the most sensitive indicators of Ni exposure, areas of focal necrosis and altered bile ducts were observed in livers of treated fish. Histological alterations were seen throughout the posterior kidneys, in glomeruli, tubules, collecting ducts, and hematopoietic tissue, in fish fed 100 or 1 000 µg Ni/g. The frequency (%) of altered distal tubules and fields of views with alterations in kidneys increased with the dose and duration of exposure. The CONTAM Panel derived a NOAEL of 0.2 mg/kg b.w. per day (10 mg Ni/kg feed) for fish from this study.

Zebrafish were chronically fed either a diet containing 116 Ni mg/kg or a control diet (3.7 mg Ni/kg) for 80 days (Alsop et al., 2014). Ni-exposed male fish, but not female fish, were significantly smaller (26 %) compared to control fish after 80 days exposure to Ni. Furthermore, total egg production was decreased by 65 % in the Ni-treated female fish at day 75–78 of the study. Ni exposure resulted in significant Ni accumulation in brain, vertebrae and gut (44 %, 34 % and 25 % increase, respectively, compared to control fish).

In Table 12 toxicity data on fish are summarised.

Table 12: Relevant nickel (Ni) toxicity studies with fish

Species Doses Exposure duration	No observed adverse effect levels (NOAELs)	Ni levels associated with adverse effects	Adverse effects observed	References
Major carps ca. 73 mg Ni/kg feed (given as NiCl ₂); 12 weeks	< 2.2 mg Ni/kg b.w. per day	73 mg Ni/kg feed corresponding to 2.2 mg Ni/kg b.w. per day (based on a feed intake of 0.020 kg feed per kg b.w. per day)	Decrease of weight gain, fork length and feed intake	Javed (2013)
Lake whitefish 1 000 and 10 000 mg Ni/kg feed (given as NiSO ₄); 18 days	< 20 mg Ni/kg b.w. per day	1 000 mg Ni/ kg feed corresponding to 20 mg Ni/kg b.w. per day (based on a feed intake of 0.020 kg feed intake/kg b.w. per day)	Histopathological alterations in the kidney and of metabolic parameters	Ptashinsky et al. (2001)
Lake whitefish 10, 100 and 1 000 mg Ni/kg feed (given as NiSO ₄); 10, 31 and 104 days	10 mg Ni/kg feed corresponding to 0.2 mg Ni/kg b.w. per day (based on a feed	100 mg Ni/kg feed corresponding to 2 mg Ni/kg b.w. per day (based on a feed intake of 0.020 kg	Histopathological alterations in kidney	Ptashinsky et al. (2002)

Species Doses Exposure duration	No observed adverse effect levels (NOAELs)	Ni levels associated with adverse effects	Adverse effects observed	References
	intake of 0.020 kg feed per kg b.w. per day)	feed/kg b.w. per day)		
Zebrafish 116 mg Ni/kg feed (given as NiSO ₄); 80 days	< 2.3 mg Ni/kg b.w. per day	116 mg Ni/kg feed corresponding to 2.3 mg Ni/kg b.w. per day (based on a feed intake of 0.020 kg feed per kg b.w. per day)	Reduced growth in males. Reduced total egg production in females.	Alsop et al. (2014)

b.w.: body weight; NOAEL: no observed adverse effect level.

7.3.4. Horses

The CONTAM Panel has not identified any studies on toxicity of Ni in horses.

7.3.5. Dogs

Ni sulphate hexahydrate was administered orally via the diet to Beagle dogs for two years at doses of 0, 100, 1 000 and 2 500 mg Ni/kg food (corresponding to 0, 1.8, 18 and 45 mg Ni/kg b.w. per day) (Ambrose et al., 1976). All dogs survived the 2-year experimental period. During the first three days, all six dogs from the highest dose group vomited, usually within 1 hour. On the fourth day, they returned to the control diet. All but one dog readjusted within three days. The one dog readjusted after parenteral feeding and intravenous fluids. At the start of the second week, five of the dogs were placed on 1 500 mg Ni/kg food and the sixth dog was included at the start of the sixth week. This level of Ni was well tolerated. At two-week intervals the diet level of Ni was raised to 1 700, 2 100 and 2 500 mg Ni/kg food, respectively, with no further evidence of emesis, salivation or gastrointestinal irritation. Decreased b.w. was observed at the highest dose. There was a tendency toward lower haematocrit and haemoglobin values at the highest dose, suggestive of a simple hypochromic anaemia. Marked polyuria was noted in two dogs at the highest dose. Relative kidney and liver weights were higher at the highest dose. At high dose, all dogs showed lung lesions (multiple subpleural peripheral cholesterol granulomas, bronchiolectasis, emphysema and focal cholesterol pneumonia) and two dogs had granulocytic hyperplasia of the bone marrow. The CONTAM Panel identified a NOAEL of 1 000 mg Ni/kg food corresponding to 18 mg Ni/kg b.w. per day. The results from this study are presented also in Table 13.

Table 13: Relevant nickel (Ni) toxicity data on dogs

Species Doses Exposure duration	No observed adverse effect levels (NOAELs)	Ni levels associated with adverse effects	Adverse effects observed	References
Beagle dogs; 0–2 500 mg Ni sulphate hexahydrate/kg feed; 2 years	18 mg Ni/kg b.w. per day	45 mg Ni/kg b.w. per day	Vomiting, polyuria, lung lesions, granulocytic hyperplasia of the bone marrow	Ambrose et al. (1976)

b.w.: body weight.

7.3.6. Cats

The CONTAM Panel has not identified any studies on toxicity of Ni in cats.

7.3.7. Conclusions on toxic effects in animals

There are only rather limited data available on oral toxicity in livestock species, fish and dogs. Only a few of these are suitable for derivation of NOAELs. No data are available for horses and cats.

The main adverse effects seen in cattle, pigs and rabbits are:

- (i) reduced feed consumption and body weight (growth);
- (ii) reduced relative organ weights, and
- (iii) histopathological alterations in kidney and liver and altered blood parameters.

In chickens, more severe toxic effects were observed including increased mortality and malformations.

In fish, decreases in b.w. gain and histopathological alterations were observed. In dogs, Ni caused a variety of effects as already described above in this section.

8. Risk characterisation for livestock, companion animals and fish

As described in the previous Section 7 only a small number of relevant Ni toxicity studies are available for livestock species. These were repeat dose toxicity studies of relatively short duration where Ni has been added to feed. One study in poultry involved administering Ni-containing feed to both parent hens and cocks and their offspring. In dogs, a chronic toxicity study is available. All the available studies have been carried out with divalent Ni (i.e. chloride, sulphate or carbonate). A major limitation in the interpretation of these studies is that most of them were not designed for deriving NOAELs.

For cattle, only one relevant study (O'Dell et al. 1970b) was identified. The CONTAM Panel derived a NOAEL of 1.34 mg/kg b.w. per day (LOAEL of 5.34 mg/kg b.w. per day for reduced feed intake and growth), to be used for risk characterisation.

For pigs, the CONTAM Panel identified a NOAEL of 12.8 mg/kg b.w. per day (LOAEL of 18.6 mg/kg b.w. per day) for reduced feed intake and body weight gain, to be used for risk characterisation in a six weeks study with piglets (Kirchgessner and Roth, 1977).

For rabbits, a NOAEL of 3.75 mg/kg b.w. per day (LOAEL of 37.8 mg/kg b.w. per day based on reduced relative weights of liver, kidneys and ovaries, and reduced ovary functionality) established in a five weeks study with adult female rabbits (Bersényi et al., 2004) was selected for use in risk characterization by the CONTAM Panel. However, the CONTAM Panel noted that the available data do not provide robust information on reproductive effects. This is of importance as some data indicate possible effects on the reproductive system, in particular the findings of considerable accumulation of Ni in ovaries of female rabbits observed in conjunction with reduced weight and functionality of the ovaries (Bersényi et al., 2004).

For ducks, a 90-day study with mallard ducklings (Cain and Pafford, 1981) was the only identified study. The CONTAM Panel identified a NOAEL of 9.4 mg/kg b.w. per day (a LOAEL of 37.6 mg/kg b.w. per day was derived based on findings of decreased bone density), for use in the risk characterisation.

For fish the only NOAEL identified (Ptashinsky et al., 2002) of 0.2 mg Ni/kg b.w. per day (LOAEL of 2 mg/kg b.w. per day for histopathological alterations in the kidney) was used for risk characterisation.

For dogs, the NOAEL of 18 mg/kg b.w. per day (LOAEL of 45 mg/kg b.w. per day based on vomiting polyuria, lung lesions and bone marrow hyperplasia) seen in a two year study with Beagles was used for the risk characterisation.

For chickens, several studies are available but overall, no reliable NOAEL could be derived from these as in most studies adverse effects were observed already at lowest doses tested. In absence of a NOAEL, the lowest LOAEL of 3.0 mg Ni/kg b.w. per day based on slightly reduced growth, slightly reduced relative weights of livers and testicles and mild pathological liver focal fatty infiltration together with a decrease of specific blood parameters in the study of Bersényi et al. (2004) was used to characterise the risk. The results obtained in the study by Trüpschuch et al. (1996) indicate an increase of Ni toxicity for chicken offspring following exposure of parent animals. Therefore the LOAEL derived on the basis of a study following a different and possibly less sensitive study design (Bersényi et al., 2004) should be considered with some prudence.

No NOAELs/LOAELs could be derived for sheep, goats, turkeys, horses and cats as no relevant toxicity studies could be identified.

The NOAELs/LOAELs identified as relevant have been compared with exposures to Ni from feed assuming maximum concentrations of Ni in feed from good manufacturing practice in these feed materials (50 mg/kg) and a maximum inclusion rate of 5 % hydrogenated oils in the compound feed (see Section 6.2 on exposures derived from concentrations in feed materials). The CONTAM Panel considered this as a worst-case scenario.

Resulting estimates of mean upper bound chronic exposure to Ni from feed were 0.06 mg/kg b.w. per day for cattle (i.e. 'Beef: intensive cereal', see Table 7, Section 6.2.1), 0.18 mg/kg b.w. per day for pigs and ducks ('Pig starter' and 'Ducks for fattening', see Table 8, Section 6.2.2), 0.01 mg/kg b.w. per day for fish (see Section 6.2.4), 0.04 mg/kg b.w. per day for dogs (see Table 9, Section 6.2.3) and 0.20 mg/kg b.w. per day for chickens ('Chickens for fattening', see Table 8, Section 6.2.2).

In Table 14 worst-case exposure estimates are presented together with NOAELs/LOAELs for the different animal species.

Table 14: NOAELs/LOAELs and chronic exposure levels for different livestock species, fish and dogs

Livestock species	NOAEL (mg Ni/kg b.w. per day)	LOAEL (mg Ni/kg b.w. per day)	Estimated chronic exposure levels (UB) mg Ni/kg b.w. per day	NOAEL exceeding chronic exposure level
Cattle	1.34 ^(a)	5.34 ^(a)	0.06	22 fold
Pigs	12.8 ^(b)	18.6 ^(b)	0.18	71 fold
Rabbits	3.75 ^(c)	37.8 ^(c)	0.23	16 fold
Ducks	9.4 ^(d)	37.6 ^(d)	0.18	52 fold
Fish	0.2 ^(e)	2.0 ^(e)	0.01	20 fold
Dogs	18 ^(f)	45 ^(f)	0.04	450 fold
Chicken	n.a.	3.0 ^(g)	0.20	15 fold ^(h)

b.w.: body weight; Ni: nickel; NOAEL: no observed adverse effect level; UB: upper bound; n.a.: not available.

(a): O'Dell et al. (1970b).

(b): Kirchgessener and Roth (1977);

(c): Bersényi et al. (2004);

(d): Cain and Pafford (1981);

(e): Ptashinsky et al. (2002);

(f): Ambrose et al. (1976);

(g): Bersényi et al. (2004);

(h): In the absence of a NOAEL the comparison has been made with the LOAEL.

When the NOAELs derived from the available toxicity studies are compared with the estimated chronic exposures to Ni (see Table 14), it is evident that exposure estimates to Ni are considerably lower than the relevant NOAELs. Taking into account the conservative approach adopted in the present opinion for estimating exposures, the CONTAM Panel concludes that, despite the considerable limitations of the available data, any impact of exposure to Ni in feed of cattle, pigs, rabbits, ducks,

fish, chicken and dogs is unlikely. For chickens, only a LOAEL could be used for risk characterisation. However, since the worst case exposure estimate for this species is around 15 times lower than the LOAEL, which is based only on minor alterations of study parameters investigated, it is unlikely that any Ni in feed mediated health effects in this species occur.

Mean UB exposure estimates for animal species for which no relevant toxicological data were available were 0.08 mg/kg b.w. per day for sheep ('Sheep: lactating', see Table 7, Section 6.2.1), 0.16 mg/kg b.w. per day for goats ('Goats: lactating', see Table 7, Section 6.2.1), 0.04 mg/kg b.w. per day for horses (see Table 7, Section 6.2.1), 0.11 mg/kg b.w. per day for turkeys (see Table 8, Section 8) and 0.04 mg/kg b.w. per day for cats (see Table 9, Section 6.2.3).

Although for turkeys no NOAEL/LOAEL is available for adverse effects of Ni it can be assumed that based on the considerable margin between worst case exposure levels and the NOAELs and LOAELs derived in other poultry species (i.e. chickens and ducks), any adverse effect in turkeys via Ni in feed is unlikely. Similarly, comparing the worst case exposure levels derived for goats, sheep and horses with the NOAEL for Ni in cattle (see Table 14), the CONTAM Panel concluded that any adverse effects of Ni mediated through the presence of Ni in feed in these species are unlikely.

No information was identified on specific toxicity of Ni in cats. However, the worst-case exposure level derived for this species (0.04 mg/kg b.w. per day) is about 450 times lower than NOAEL derived for dogs (18 mg/kg b.w. per day). Therefore any Ni in pet food mediated adverse effects are unlikely to occur in cats.

The CONTAM Panel noted that the data submitted by FEDIOL and shortly described in Section 4.3 suggest that actual values for Ni content in vegetable oils are substantially lower than the maximum concentrations of Ni in feed from good manufacturing practice and therefore support the conclusions of the animal risk assessment.

9. Human health risk assessment related to nickel dietary exposure from foods of animal origin

9.1. Nickel occurrence data in foods of animal origin

Within the EFSA Food classification and description system for exposure assessment (FoodEx) (EFSA, 2011a), there are several food groups that contain foods of animal origin. Table 15 shows the Ni occurrence values (mean and 95th percentile) for these foods ($n = 3\,713$). It can be seen that foods are mainly represented in three groups, in terms of the number of available data, namely 'Meat and meat products' ($n = 2\,169$), 'Fish and other seafood' ($n = 718$) and 'Milk and dairy products' ($n = 584$). Other foods from animal origin were in the groups 'Animal fats', 'Eggs and egg products' and 'Food for infants and small children'. Samples were collected between 2003 and 2012 in seven different European countries, with Slovakia and Germany the main sampling countries with 1 788 and 1 553 samples, respectively.

Of the 3 713 samples available, 65 % of them were left-censored data; this percentage was similar across the three main food groups. Overall, foods of animal origin possess lower levels of Ni compared to other foods that are well-known sources of this mineral, such as chocolate, legumes, nuts or oilseeds (EFSA CONTAM Panel, 2015). Nevertheless, in the reported data on foods of animal origin there were few samples that presented high values of Ni; these samples mainly referred to meat and meat products, in particular to some edible offal (Table 15). For certain food commodities no occurrence data were available. To avoid underestimation of the dietary exposure, different occurrence values were assigned to these commodities. Details for each specific case are described in the footnotes to Table 15.

Certain foods that might contain variable amounts of foods of animal origin (mainly milk) such as ice creams, cereal-based food for infants and young children, desserts or chocolate with milk, are not

included. In general, in these foods the amount of food of animal origin is minor compared to the other components, or data on its presence were incomplete or absent.

Table 15: Occurrence data on nickel (Ni) ($\mu\text{g/kg}$) for foods of animal origin at the FoodEx level used to estimate chronic dietary exposure

Groups ^(a)	Food commodities ^(b)	N	% LC ^(c)	Mean		95th percentile ^(e)	
				LB ^(d)	UB ^(d)	LB ^(d)	UB ^(d)
Animal fats	<i>Butter</i>	61	56	78	92	290	290
	<i>Pork lard (Schmaltz)</i>	65	58	330	330	360	360
Eggs and egg products	Eggs and egg products	115	74	38	57	180	180
Fish and other seafood	Fish meat	545	69	56	84	210	260
	Fish offal	17	6	99	99	–	–
	Crustaceans	69	39	43	180	130	580
	Water molluscs	51	2	390	390	–	–
	Fish products ^(f)			77	110	330	390
	Unspecified fish and other seafood	32	88	8.2	32	–	–
Meat and meat products (including edible offal)	Livestock meat	629	61	96	120	330	330
	Poultry	231	76	63	99	130	210
	Game birds ^(g)			63	99	130	210
	Game mammals	264	64	170	190	580	580
	Preserved meat	8	50	18	26	–	–
	Sausages	277	59	150	240	170	610
	Meat specialities ^(h)			190	240	310	510
	Pastes, pâtés and terrines ^(h)			190	240	310	510
	Mixed meat ^(h)			190	240	310	510
	Edible offal, game animals	45	49	140	160	–	–
	<i>Beef kidney</i>	18	72	17	51	–	–
	<i>Beef liver</i>	303	57	120	140	410	410
	<i>Giblets (chicken, turkey, duck, goose)</i>	57	91	4.6	49	–	–
	<i>Mutton/lamb liver</i>	19	42	1 300	1 300	–	–
	<i>Pork kidney</i>	102	81	30	190	190	510
	<i>Pork liver</i>	187	83	970	1 100	190	510
	<i>Other edible offal, farmed animals⁽ⁱ⁾</i>			350	420	320	510
Milk and dairy products	Cheese	145	59	90	110	320	320
	Fermented milk products	58	85	7.7	76	–	–
	Liquid milk	355	67	21	31	91	91
	Dried milk ⁽ⁱ⁾			230	350	990	990
	Evaporated milk ⁽ⁱ⁾			61	94	270	270
	Condensed milk ⁽ⁱ⁾			61	94	270	270
Food for infants and small children	Yoghurt, cheese and milk-based dessert for infants and young children ^(k)			7.7	76	–	–

(a): Food samples were grouped at FoodEx level 1 to better explain their contribution to the dietary exposure to Ni;

(b): Within each food group, and depending on their reported occurrence values, the samples were grouped at FoodEx level 1 (bold), level 2 (normal), level 3 (italics), before being linked with the EFSA Comprehensive Food Consumption Database;

(c): Percentage of left-censored data;

(d): LB = Lower bound, UB = Upper bound;

(e): The 95th percentile for samples with less than 60 observations is not shown as the results may not be statistically robust (EFSA, 2011b);

(f): Mean value obtained from the average concentration of the food commodities grouped at FoodEx level 1;

(g): The occurrence values reported for 'Poultry' were used;

- (h): The occurrence values reported for all samples of ‘Meat and meat products (including edible offal)’ at FoodEx level 1 were used;
- (i): Mean value obtained from the average concentration of the available food commodities grouped at FoodEx level 2;
- (j): Occurrence values for ‘Dried milk’ were calculated multiplying the samples of ‘Liquid milk’ by a factor of 11, and by a factor of three to obtain the occurrence values of ‘Evaporated milk’ and ‘Condensed milk’;
- (k): Since only one sample was reported, the occurrence value reported for ‘Fermented milk’ was used. All values presented are rounded to two significant figures.

9.2. Human dietary exposure to nickel from food of animal origin

9.2.1. Chronic exposure

To calculate chronic dietary exposure to Ni, food consumption and body weight data at the individual level were accessed in the Comprehensive Database (Huybrechts et al., 2011). The mean and the high (95th percentile) chronic dietary exposure was calculated by combining Ni mean occurrence values (Table 15, pooled European occurrence data) with the average daily consumption for each food of animal origin at individual level in each dietary survey.

Summary statistics of chronic dietary exposure estimates are shown in Table 16. The highest mean and 95th percentile dietary exposure to Ni considering only food of animal origin was estimated in ‘Toddlers’ (Table 16). In addition, the average contribution of food of animal origin to the total dietary exposure to Ni was also calculated (Appendix E, Table E1).

Table 16: Summary statistics of the chronic exposure assessment ($\mu\text{g/kg b.w. per day}$) to nickel (Ni) from food of animal origin across European dietary surveys

Mean dietary exposure from food of animal origin ($\mu\text{g/kg b.w. per day}$)						
	LB			UB		
	Min	Median	Max	Min	Median	Max
Infants	0.4	— ^(a)	1.7	1.4	— ^(a)	2.6
Toddlers	0.9	1.4	2.8	1.4	2.4	3.8
Other children	0.5	0.9	1.5	0.8	1.5	2.5
Adolescents	0.3	0.5	0.6	0.4	0.8	0.9
Adults	0.3	0.4	0.5	0.4	0.6	0.8
Elderly	0.3	0.3	0.4	0.4	0.5	0.6
Very Elderly	0.2	0.3	0.4	0.4	0.4	0.6
95th percentile dietary exposure from food of animal origin ($\mu\text{g/kg b.w. per day}$) ^(b)						
	LB			UB		
	Min	Median	Max	Min	Median	Max
Infants	1.3	— ^(c)	— ^(c)	— ^(c)	— ^(c)	5.4
Toddlers	1.6	— ^(a)	3.1	2.5	— ^(a)	5.5
Other children	1.0	1.7	3.7	1.7	2.6	4.9
Adolescents	0.6	1.0	1.1	0.9	1.4	1.7
Adults	0.5	0.7	1.2	0.7	1.0	1.5
Elderly	0.5	0.6	0.8	0.8	0.9	1.0
Very Elderly	0.5	— ^(a)	0.7	0.6	— ^(a)	0.9

b.w.: body weight; LB: lower bound; Max: maximum; Min: minimum; UB: upper bound.

(a): Not calculated since estimates were only available from less than six dietary surveys.

(b): The 95th percentile estimates obtained on dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b). Those estimates were not included in this table.

(c): Not calculated since estimates were only available from one dietary survey. Estimates were rounded to one decimal place.

Overall, in the young population (‘Infants’, ‘Toddlers’ and ‘Other children’) the main contributor within the foods of animal origin was ‘Milk and dairy products’, in particular in infants and toddlers. Also important was the food group ‘Meat and meat products’ that in few surveys of ‘Other children’ became the main contributor within the foods of animal origin. The role of ‘Milk and dairy products’

in the dietary exposure to Ni is mainly explained by its importance in the diet of the young population since this food group possesses relatively low levels of Ni. In the older population ('Adolescents', 'Adults', 'Elderly' and 'Very elderly'), 'Meat and meat products' gained importance and became the main contributor within the foods of animal origin in most of the dietary surveys, with a median contribution of 50.1 % among the foods of animal origin (range 35.6–72.4 %). The subgroups 'Livestock meat' and 'Sausages' represented more than 50 % of the contribution of 'Meat and meat products' (range 53–88 % across 'Adults'). The contribution of the remaining food groups ('Fish and other seafood', 'Animal fats', 'Eggs and egg products' and 'Food for infants and small children') was overall insignificant, with the exception of 'Fish and other seafood' which, in certain populations, reached average contributions around 25 % within the foods of animal origin.

Despite their importance within the foods of animal origin in the adult population, 'Meat and meat products' were far from being a main contributor to the total dietary exposure to Ni, which are 'Grain and grain-based products', 'Non-alcoholic beverages (except milk-based beverages)', 'Sugar and confectionary', 'Legumes, nuts and oilseeds', and 'Vegetables and vegetable products (including fungi)'.

Figure 2 shows for 'Adults' and for 'Toddlers' the median average contribution of 'Milk and dairy products' and 'Meat and meat products' to the total dietary exposure to Ni together with the most important contributors to exposure. The figure shows the median across dietary surveys.

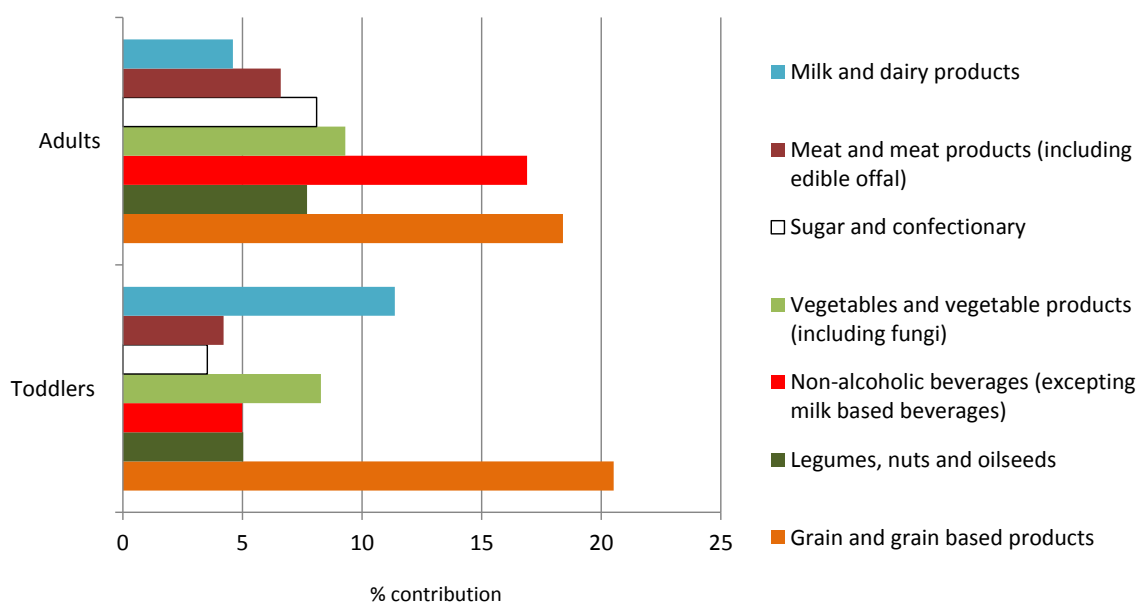


Figure 2: Median (across dietary surveys) of the average contribution of different food groups to the dietary exposure to nickel (Ni) in the age classes 'Toddlers' and 'Adults'

Without considering infants, for which only two dietary surveys were available, the average contribution of the foods of animal origin (at the LB estimations) to the mean dietary exposure across the different age classes ranged as follows: 'Toddlers' (13.0–29.1 %, median = 17.6 %), 'Other children' (9.4–23.8 %, median = 13.4 %), 'Adolescents' (9.9–18.5 %, median = 12.1 %), 'Adults' (10.1–17.0 %, median = 13.9 %), 'Elderly' (11.6–15.9%, median = 14.2 %), 'Very elderly' (11.5–15.1 %, median = 12.8 %). It can be seen that the average contribution of the foods of animal origin to the mean chronic dietary exposure to Ni (at the LB estimations) ranged between 9.4 % (lowest LB in 'Other children') and 29.1 % (highest LB in 'Toddlers').

Detailed description of the average contribution of foods of animal origin for each dietary survey is given in Appendix E, Table E1. The average contribution of the foods of animal origin to the dietary

exposure to Ni in the highly exposed population was, overall, similar to that observed in the whole population.

9.2.2. Acute exposure

In the EFSA opinion on Ni in food (EFSA CONTAM Panel, 2015) average acute exposure estimations did not differ substantially from those calculated for the chronic exposure. Based on the outcome of the probabilistic acute exposure estimates presented in the CONTAM opinion on Ni in food (EFSA CONTAM Panel, 2015), the CONTAM Panel considered that there was no need to perform a full probabilistic dietary exposure assessment specifically for food of animal origin for the present opinion. Nevertheless, for the current opinion single point-estimates of the acute dietary exposure were calculated combining high consumption (95th percentile) for selected foods of animal origin with 95th percentile occurrence values (Table 16). ‘Toddlers’ (age class with the highest chronic dietary exposure) and ‘Adults’ were selected as representative age classes of the young and adult population, respectively. The selected foods in the adult population were liquid milk, livestock meat and fish meat as the main representatives of ‘Milk and dairy products’, ‘Meat and meat products’ and ‘Fish and other seafood’, respectively. Based on the outcome of the chronic exposure assessment, the single point-estimate in ‘Toddlers’ was focused only on liquid milk.

In ‘Adults’ (70 kg default b.w.), the selection of both 95th percentile consumption and occurrence values of liquid milk (300 mL), livestock meat (200 g) and fish meat (200 g) would lead to dietary exposure estimations of 0.4 µg/kg b.w. per day, 0.9 µg/kg b.w. per day and 0.6 µg/kg b.w. per day, respectively. For ‘Toddlers’ (12 kg default b.w.), the combination of both 95th percentile consumption and occurrence values of liquid milk (250 mL) would lead to dietary exposure estimates of 1.9 µg/kg b.w. per day.

9.3. Risk characterisation for humans

9.3.1. Chronic effects

In its recent opinion on Ni in food the CONTAM Panel established a TDI of 2.8 µg/kg b.w. per day (EFSA CONTAM Panel, 2015). The mean chronic dietary exposure to Ni from foods of animal origin, across the different dietary surveys ranged, in adolescents, adults, elderly and very elderly, from 0.3 µg/kg b.w. per day (minimum LB, adolescents, adults, elderly, very elderly) to 0.9 µg/kg b.w. per day (maximum UB, adolescents), which are well below the TDI. However, the mean chronic dietary exposure to Ni from foods of animal origin in the age classes infants, toddlers and other children ranges from 0.4 (minimum LB, infants) µg /kg b.w. per day to 3.8 µg /kg b.w. per day (maximum UB, Toddlers) and is, in several cases, close to or above the TDI when considering the UB exposure of these age classes.

In the population highly exposed to Ni through the consumption of food of animal origin, the 95th percentile estimates varied in the young population from 1.0 µg/kg b.w. per day (minimum LB, other children) to 4.9 µg/kg b.w. per day (maximum UB, other children). Although the LB estimations remain in many cases below the TDI, the UB estimations are in most of the dietary surveys above the TDI. As observed for the mean dietary exposure, the older population (adolescents, adults, elderly and very elderly) showed lower exposure than the young population with estimates that varied between 0.3 µg/kg b.w. per day (minimum LB, elderly and very elderly) and 1.7 µg/kg b.w. per day (maximum UB).

Based on the above-mentioned values, the CONTAM Panel concluded that in the average population the current levels of exposure to Ni considering only foods of animal origin might be of potential concern in the young population, in particular in toddlers. When assessing the highly exposed population (95th percentile) the CONTAM Panel concluded that the exposure to Ni considering only foods of animal origin might be of potential concern not only in toddlers, but also in the age class ‘Other children’.

9.3.2. Acute effects

In its opinion on Ni in food the EFSA CONTAM Panel established a BMDL₁₀ of 1.1 µg Ni/kg b.w. for hypersensitivity reactions for a MOE approach. In that opinion, all the MOEs calculated from the acute dietary exposure levels were considerably below 10 for all age groups both for the estimated mean and 95th percentile exposure levels. Based on this, the CONTAM Panel concluded that at the current levels of dietary exposure to Ni, Ni-sensitized individuals were at risk of developing eczematous flare up skin reactions (EFSA CONTAM Panel, 2015).

In the context of the current opinion the combination of high consumption (95th percentile) for representative foods of animal origin with 95th percentile occurrence values, resulted in acute dietary exposure estimations in ‘Adults’ of 0.4 µg/kg b.w. per day, 0.9 µg/kg b.w. per day and 0.6 µg/kg b.w. per day for liquid milk, livestock meat and fish meat respectively. In ‘Toddlers’, the use of both 95th percentile consumption and occurrence values of liquid milk led to dietary exposure estimates of 1.9 µg/kg b.w. per day. These single-point estimates of the acute dietary exposure based on the individual consumption of food of animal origin show MOEs values below 10 for the two age classes considered.

Therefore, the CONTAM Panel concludes that Ni-sensitized individuals are already at risk of developing eczematous flare up skin reactions from only the consumption of food of animal origin.

10. Risks to the environment from the presence of nickel in feed

As discussed in Section 1.4, Ni occurs naturally in the environment, largely as a result of volcanic activity and industrial and anthropogenic processes. In soils, levels of Ni vary widely (ATSDR, 2005). In most agricultural soils concentrations range from 3 to 1 000 mg/kg (EFSA CONTAM Panel, 2015), and a mean of 23.9 mg Ni/kg has been reported.²⁵ Ni is not intentionally added to feed, but is naturally present in feeds and may occur as a process-contaminant (see Section 4). Since Ni in feed has a low bioavailability, most of the Ni consumed is returned to soils in manures.

The Ni contents of livestock manures are largely a reflection of the Ni content of livestock feed. Based on data provided by Member States, the EC (2001) has reported ranges for the Ni contents of livestock manures. Mean concentrations reported by Nicholson et al. (1999) for livestock manures in England and Wales generally fall within these ranges (Table 17).

Table 17: Typical nickel (Ni) content of livestock manures (mg/kg dry solids)

Type of manure	Mean values
Pig slurry	4.88 ^(a)
Pig slurry	3.2–17 ^{(b),(c)}
Pig FYM	2.67 ^(a)
Pig FYM	5 ^(b)
Dairy slurry	8.48 ^(a)
Dairy slurry	3.3–14 ^{(b),(c),(d)}
Dairy FYM	3.92 ^(a)
Dairy FYM	1–14 ^{(b),(c)}
Beef slurry	9.01 ^(a)
Beef FYM	4.70 ^(a)
Sheep FYM	5.94 ^(a)
Layer manure	7.20 ^(a)
Layer manure	4.9–17 ^{(b),(c)}
Broiler litter	2.01 ^(a)

FYM: Farmyard Manure.

(a): Nicholson et al. (1999);

(b): EC (2001);

(c): Numbers give range of values instead of mean;

²⁵ www.nickelinstitute.org

(d): reported as 'cattle manure'.

Based on similar levels of Ni in livestock manures, and typical application rates of manure to soils, Nicholson et al. (1999) estimated that the total amount of Ni deposited on agricultural soils in England and Wales from livestock manures and slurries was approximately 32 g/ha.

Figure 3 illustrates the relative contributions of Ni from different sources to agricultural land, and the relatively small contribution from livestock manures. The CONTAM Panel recognises that farming practices vary within the EU, but these figures provide an indication of the scale of the input from livestock.

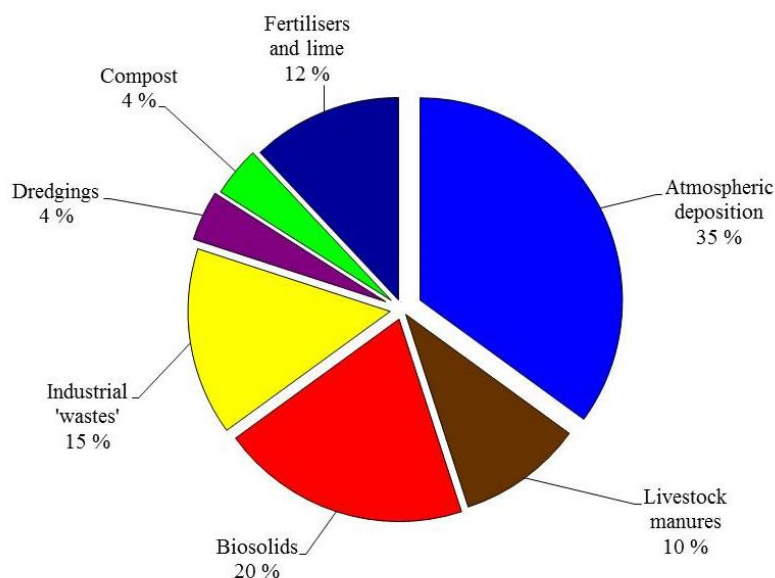


Figure 3: Distribution of sources of nickel onto agricultural land

While a comprehensive assessment of the environmental impact of Ni in livestock manures is outside the scope of this opinion, these data would suggest that the Ni in feed, and subsequently in manure, is not a major contributor of Ni deposited onto agricultural soils or to the environment.

11. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of the risks for public and animal health and the environment related to the presence of Ni in feed has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). According to the guidance provided by the EFSA opinion (2006) the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model and model input (parameters).

11.1. Assessment objectives

The objectives of the assessment were specified in the terms of reference.

11.2. Occurrence data and exposure assessment in food and feed

The occurrence data used for the exposure assessment of animals came mainly only from one country (Slovakia). Since these data may not represent the general situation in the EU, this contributes to the overall uncertainty.

The animal risk assessment is hampered by limited representative feed consumption data for livestock and fish across Europe. As a result, there is considerable uncertainty regarding the total dietary exposure to Ni in the animal risk assessment. Due to the absence of data on the Ni content of hydrogenated oils and fats used in animal diets, a 'worst-case' approach was adopted which is likely to have over-estimated exposure in most cases, although the extent of this is uncertain.

The robustness of the alternative approach for estimating exposure, namely that based on compound feeds, was limited due to the lack of data for individual animal categories and the need for aggregation across all species.

Although it is known that livestock take in Ni from soil and water, it was not possible to quantify exposure from these routes. Under some foraging conditions the intake of Ni from soil might be substantial and will be influenced by factors such as soil Ni content, herbage type and density, grazing intensity and rainfall. Therefore, in those cases exposure calculated only from feed is likely to have underestimated total exposure to Ni.

Because no validated methods exist for Ni speciation in feed, occurrence and exposure data were mostly reported as total Ni. No data were available for metallic Ni in feed or animal material. This may add to the overall uncertainty.

The type and extent of feed processing may have an influence, due to migration of metallic Ni from the stainless steel present in the processing equipment. Additionally, metallic Ni and Ni oxides are used as catalysts in the hydrogenation process of oils and thus might enter feed in significant amounts. Due to the lack of data, the CONTAM Panel was not able to quantitatively assess the contribution of feed processing. This may add to the overall uncertainty.

The occurrence data on Ni in animal derived food presented in the present opinion and used for exposure assessment are extracted from the data set used for the opinion on Ni in food (EFSA CONTAM Panel, 2015). Therefore the uncertainties associated with the occurrence data and the dietary exposure of food of animal origin, addressed in that assessment are also relevant for the present opinion.

11.3. Other uncertainties

Only a small number of studies on Ni toxicity are available and only for a few livestock species, fish, and dogs, and no toxicity data are available on horses and cats. Most of the studies were not designed for deriving NOAELs/LOAELs. In some cases, inadequate reporting was also noted. With the exception of a single study in chickens, the studies do not cover reproductive toxicity although some data in rabbits and poultry indicate possible effects on the reproductive system.

None of the toxicity studies with fish was designed to derive NOAELs/LOAELs. The endpoints used for risk characterisation were histological alterations in kidney that were not quantified, which adds to the uncertainty.

The fact that all the toxicity data have been obtained by exposing animals to soluble divalent Ni while in practice, exposure of these animals is to metallic Ni or to Ni forms likely to occur in biological systems (i.e. Ni complexed or bound to biomolecules) adds to the uncertainty.

Since the health based guidance values used for characterisation of the risk to human health from Ni in animal derived food have been adopted from the CONTAM Panel opinion on Ni in food (EFSA CONTAM Panel, 2015) all uncertainties incurred with their derivation also apply for the present assessment.

11.4. Summary of uncertainties

In Table 18 a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty leads to over/underestimation of the resulting risk.

Table 18: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the nickel (Ni) in feed

Sources of uncertainty	Direction ^(a)
Occurrence data in feed stemmed mainly from one country thus they may not well represent the general situation in the European Union	+/-
Representativeness of feed consumption data in livestock is limited	+/-
In the absence of information on Ni content in compound feed highest permitted maximum levels of Ni were assumed for animal exposure assessment	+
Ni levels in water and soil have not been considered for animal risk assessment although they might contribute to total Ni intake in animals	-
Occurrence in feed is only reported as total Ni	+/-
Only limited information on Ni toxicity is available for livestock animals, fish and cats	+/-
Lack of reproduction toxicity studies in certain livestock species	+/-
Lack of data on Ni in manure and environmental fate of Ni in soil	+/-

(a): +: uncertainty with potential to cause over-estimation of exposure/risk; -: uncertainty with potential to cause under-estimation of exposure/risk.

Overall, the CONTAM Panel concluded that the impact of the uncertainties on the animal health risk assessment is small and that the risk assessment is more likely to overestimate than to underestimate the risks.

The uncertainties associated with the human health risk assessment addressed in the opinion on Ni in food (EFSA CONTAM Panel, 2015) are also relevant to this opinion.

The CONTAM Panel concluded that the impact of the uncertainties on the assessment of the contribution to environmental Ni load as a result of its presence in manure is small.

CONCLUSIONS AND RECOMMENDATION

CONCLUSIONS

General

- Nickel (Ni) is found in all environmental compartments and is ubiquitous in the biosphere. Its presence in feed can arise from both natural and anthropogenic sources.
- Ni in feed generally occurs in the divalent form, its most stable oxidation state, but may also occur in other oxidation states
- Feed might also contain metallic Ni, since it is used as a catalyst in the production of hydrogenated vegetable oils. Other sources (e.g. migration from processing materials) may also contribute to the presence of metallic Ni in feed.

Methods of analysis

- The most common methods used to measure total Ni in feed are atomic absorption spectrometry (AAS), either flame or graphite furnace (FAAS, GFAAS), inductively coupled plasma optical emission or mass spectrometry (ICP-OES or ICP-MS).

- Currently, no validated methods exist for Ni speciation in feed.

Occurrence/Exposure

- Due to a lack of data it has not been possible to calculate the relative contributions of metallic and soluble Ni forms to the overall exposure to Ni through feed.
- Ni is naturally present in feeds, normally at levels that have no adverse effects on livestock. Levels may be increased as a result of processing, or in the case of forages, following the application of manures and sludge.
- Under certain conditions (high soil intake and/or high soil levels) soil ingestion could contribute considerably to Ni intake in the case of foraging animals, but the extent to which this occurs could not reliably be quantified.
- Estimates of exposure, based on the mean upper bound (UB) concentration levels of Ni reported for feeds, range from 5.1 (fattening beef cattle) to 61.7 (laying hens, chickens for fattening) µg/kg body weight (b.w.) per day.
- Ni catalysts are used to hydrogenate vegetable oils used as livestock feed in particular for ruminants from which trace amounts may remain in the vegetable oils. European Union (EU) feed legislation specifies the maximum content of Ni in hydrogenated vegetable oils.
- A 'worst-case' exposure assessment was undertaken, based on example rations and levels of Ni in individual feeds, and assuming a 5 % inclusion of hydrogenated vegetable oil, containing the maximum permitted concentration of 50 mg/kg Ni, in the non-forage feed. Using this approach, the highest estimated exposure was 230 µg/kg b.w. per day for rabbits, i.e. approximately three times higher than the estimate based on levels of Ni in feed.
- In practice, exposure from hydrogenated vegetable oils is likely to be substantially lower, since according to industry data the median level of Ni in hydrogenated vegetable oils is substantially lower than the permitted maximum content of 50 mg/kg that was used in the abovementioned worst case exposure assessment.
- Mean upper bound chronic exposure estimates for Ni derived from feed materials assuming a worst case of 5 % inclusion of hydrogenated vegetable oil, containing the maximum permitted concentration of 50 mg/kg Ni where 0.06 mg/kg b.w. per day for cattle, 0.18 mg/kg b.w. per day for pigs and ducks, 0.01 mg/kg b.w. per day for fish, 0.04 mg/kg b.w. per day for dogs, 0.20 mg/kg b.w. per day for chickens, 0.08 mg/kg b.w. per day for sheep, 0.16 mg/kg b.w. per day for goats, 0.04 mg/kg b.w. per day for horses, 0.11 mg/kg b.w. per day for turkeys and 0.04 mg/kg b.w. per day for cats.
- In addition to feed, animals are exposed to Ni in water. Occurrence values for Ni in tap water and published water consumption data indicate that exposure from this source is small.
- In humans, for the estimation of chronic and acute dietary exposure to Ni, considering only food of animal origin, occurrence data were mainly represented in three groups, in terms of the number of available data, namely 'Meat and meat products' (n = 2 169), 'Fish and other seafood' (n = 718) and 'Milk and dairy products' (n = 584).
- The highest chronic dietary exposure to Ni, considering only food of animal origin, was estimated in 'Toddlers', with values that ranged between 0.9–3.8 µg/kg b.w. per day (lower bound (LB)–UB) for mean dietary exposure and between 1.6–5.5 µg/kg b.w. per day (LB–UB) in the highly exposed population (95th percentile).

- Overall, in the young population ('Infants', 'Toddlers' and 'Other children') the main contributor within the foods of animal origin was 'Milk and dairy products', in particular in infants and toddlers. In the older population ('Adolescents', 'Adults', 'Elderly' and 'Very elderly'), 'Meat and meat products' became the main contributor within the foods of animal origin in most of the dietary surveys.
- When compared to the whole diet, 'Milk and dairy products' was one of the main contributors to the chronic dietary exposure to Ni in the young population, in particular in 'Toddlers'.
- Without considering infants, for whom only two dietary surveys were available, the average contribution of the foods of animal origin to the mean chronic dietary exposure to Ni (at the LB estimations) ranged between 9.4 % ('Other children') and 29.1 % ('Toddlers').
- Single point-estimates of acute dietary exposure to Ni were calculated combining high consumption (95th percentile) for selected foods of animal origin with 95th percentile occurrence values.
- In 'Adults', high consumption of liquid milk, livestock meat and fish assuming high presence of Ni led to acute dietary exposure estimations of 0.4 µg/kg b.w. per day, 0.9 µg/kg b.w. per day and 0.6 µg/kg b.w. per day, respectively. In 'Toddlers', combining high consumption and high occurrence values for liquid milk led to acute dietary exposure estimates of 1.9 µg/kg b.w. per day.

Hazard identification and characterisation

- Livestock and experimental animals absorb only a small percentage of the total oral Ni load. The extent of gastrointestinal absorption of Ni differs between animal species and depends on the amount of administered Ni, its chemical form and the composition of the vehicle used for administration (e.g. solid diet, liquid diet or water).
- Absorbed Ni is rapidly distributed to different organs and tissues in livestock and experimental animals. In livestock highest Ni concentrations are found in the kidney and in other organs/tissues such as lung, liver, muscles, testis, pancreas and spleen. Part of the absorbed Ni, is excreted in ruminant milk.
- From the available data it was not possible to determine carry-over rates from feed to food of animal origin.
- A limited number of Ni toxicity studies are available for livestock and fish. Main effects observed were (i) reduced feed consumption and body weight (growth); (ii) reduced relative organ weights; and (iii) histopathological alterations in liver and kidney and/or altered blood parameters.
- In dogs, marked polyuria, lung lesions and granulocytic hyperplasia of the bone marrow were observed upon Ni intake.
- For cattle a no observed adverse effect level (NOAEL) of 1.34 mg/kg b.w. per day was identified based on findings of reduced feed intake and growth.
- For pigs NOAEL of 12.8 mg/kg b.w. was identified based on reduced feed intake and body weight gain.

- For rabbits a NOAEL of 3.75 mg/kg b.w. per day was identified based on reduced relative weights of liver, kidneys, ovaries, reduced ovary function and altered blood parameters in female animals.
- For ducks a NOAEL of 9.4 mg/kg b.w. per day was identified based on decreased bone density.
- For fish a NOAEL of 0.2 mg Ni/kg b.w. per day was identified based on histopathological alterations in the kidney.
- For dogs a NOAEL of 18 mg Ni/kg b.w. per day was identified based on vomiting, polyuria, lung lesions and bone marrow hyperplasia at higher doses.
- For chickens a reliable NOAEL could not be identified but a lowest observed adverse effect level (LOAEL) of 3 mg/kg b.w. per day was set based on slightly reduced growth, slightly reduced relative weights of livers and testicles and mild pathological liver focal fatty infiltration together with a decrease of specific blood parameters.
- No NOAELs/LOAELs could be identified for sheep, goats, horses, turkeys and cats.

Risk characterisation

- Since the worst case chronic Ni exposure levels established for cattle, pigs, rabbits, ducks, fish and dogs are considerably lower than the respective NOAELs identified, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that there is no health concern for these species from the presence of Ni in feed, even when taking into account that, overall, the toxicity data available is limited.
- For chickens, no NOAEL could be used for risk characterisation. However, since the worst-case chronic Ni exposure level is considerably lower than the LOAEL identified in this species, which is based only on minor alterations of study parameters investigated, it is unlikely that the presence of Ni in feed is of concern for chickens.
- For turkeys no NOAEL/LOAEL is available. Based on the considerable margin between worst-case exposure levels and the NOAELs and LOAELs derived in other poultry species (i.e. chickens and ducks), it is unlikely that the presence of Ni in feed is of concern for this species.
- No NOAEL/LOAELs could be identified for goats, sheep and horses. But since worst-case Ni exposure levels estimated for these species are considerably lower than the NOAEL established for cattle, it is unlikely that the presence of Ni in feed is of concern for these species.
- No information was identified on specific toxicity of Ni in cats. However, the worst-case exposure level derived for this species is considerably lower than NOAEL derived for dogs. Therefore any Ni in pet food mediated adverse effects are unlikely to occur in cats.
- In humans, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that for the average population the current levels of chronic exposure to Ni from foods of animal origin might be of potential concern in the young population, in particular in 'Toddlers'. When assessing the highly exposed population (95th percentile) the exposure to Ni from foods of animal origin might also be of potential concern in 'Other children' as it exceeds the tolerable daily intake of 2.8 µg/kg b.w. per day.

- A BMDL₁₀ for acute oral exposure of 1.1 µg/kg b.w. per day for Ni sensitised individuals was derived in the CONTAM opinion on Ni in food. Comparing the estimates of acute dietary exposure from consumption of selected foods of animal origin for the two age classes ‘Toddlers’ and ‘Adults’ to the reference point, the margins of exposure were below 10. Therefore, the CONTAM Panel concluded that Ni-sensitized individuals are at risk of developing eczematous flare up skin reactions through the consumption of food of animal origin.
- Ni release to the environment from manure, resulting from its presence in animal feed, is not a major contributor of Ni deposited onto agricultural soils or to the environment.

RECOMMENDATION

- Studies are needed to enable determination of carry-over of Ni from feed to food products of animal origin.

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APPENDICES

Appendix A. Intakes and composition of diets used in estimating animal exposure to nickel

This Appendix gives feed intakes for different livestock and companion animals used in this Scientific Opinion to estimate exposure to nickel (EFSA CONTAM Panel, 2015). The composition of diets for each of the major farm livestock species are based on published guidelines on nutrition and feeding (e.g. AFRC, 1993; Carabano and Piquer, 1998; NRC, 2007a,b; Leeson and Summers, 2009; OECD, 2009; McDonald et al., 2011; EFSA FEEDAP Panel, 2012). They are therefore estimates of the Panel on Contaminants in the Food Chain (CONTAM Panel), and are in agreement with common practice. Based on these estimates of intake, the lower-bound (LB) and upper-bound (UB) mean concentrations of nickel (Ni) in the estimated diets for the farm livestock species and companion animals have been calculated.

A1. Feed intake

A1.1. Ruminants and horses

Live weights, feed intakes and growth rates/productivity are from AFRC (1993) and NRC (2007a,b). The live weights, feed intakes, the proportion of the daily ration that is non-forage feed and growth rates/productivity for cattle, sheep and goats used in this Scientific Opinion are given in Table A1.

Table A1: Live weights, growth rate/productivity, dry matter (DM) intake for cattle, sheep, goats and horses and the proportions of the diet as non-forage

Livestock category	Live weight (kg)	Growth rate or productivity	DM intake (kg/day)	% of diet as non-forage feed	Reference
Dairy cows; lactating ^(a)	650	40 kg milk/day	20.7	40	AFRC (1993)
Beef fattening cattle: cereal beef	400	1.4 kg/day	8.4	85	AFRC (1993)
Beef fattening cattle: forge-based	452	Moderate activity	9.0	50	NRC (2007b)
Sheep: lactating	60	Feeding twin lambs	2.8	50	AFRC (1993)
Goats: lactating	60	6 kg milk/day	3.4	25	NRC (2007a)
Goats: fattening	40	0.2 kg/day	1.5	60	NRC (2007a)
Horses	450	Moderate activity	9.0	50	NRC (2007b)

(a): Months 2–3 of lactation.

A1.2. Pigs, poultry and rabbits

Data for feed intake and live weight of pigs and poultry are from EFSA FEEDAP Panel (2012) and of ducks from Leeson and Summers (2009). The live weights and feed intakes for these animal species are presented in Table A2.

Table A2: Live weights and feed intake for pigs, poultry and rabbits

Livestock category	Live weight (kg)	Feed intake (kg DM/day)	Reference
Pigs: piglets	20	1.0	EFSA FEEDAP Panel (2012)
Pigs: fattening pigs	100	3.0	EFSA FEEDAP Panel (2012)
Pigs: lactating sows	200	6.0	EFSA FEEDAP Panel (2012)
Poultry: broilers ^(a)	2	0.12	EFSA FEEDAP Panel (2012)
Poultry: laying hens	2	0.12	EFSA FEEDAP Panel (2012)
Turkeys: fattening turkeys	12	0.40	EFSA FEEDAP Panel (2012)
Ducks: fattening ducks	3	0.14	Leeson and Summers (2009)
Rabbits	2	0.15	Carabano and Piquer (1998)

DM: dry matter.

(a): chickens for fattening.

A1.3. Fish

A wide range of diets is used for commercially farmed fish in Europe. However, the salmon feed composition described in Table A3 has been used as being representative of commercial feed producers (Berntssen et al., 2010).

Table A3: Estimated example feed composition in the diet for growing salmon

Feeds	%
Fishmeal	30.5
Wheat	13.2
Soybean meal	12.3
Maize gluten feed	11.5
Fish and vegetable oils	31.9
Minerals, vitamins, etc.	0.6

In estimating exposure, a live weight of 2 kg and feed intake of 0.04 kg/day (20 g/kg b.w. per day) has been assumed.

A1.4. Companion animals (dogs and cats)

The CONTAM Panel estimated daily intake of pet food using data compiled from six and seven different brands for dog and cat food, respectively, available on the French market (obtained from pet food stores and veterinary clinics). The assumed daily feed intakes for dogs in this Scientific Opinion are 360 g/day for a 25 kg dog, and 60 g/day for a 4 kg cat (common body weight for an adult cat for most of the European species) (J.M. Fremy, 2011, personal communication).

Appendix B. Diet composition and estimates of nickel concentration

Many livestock in the European countries, particularly non-ruminants, poultry and companion animals, are fed proprietary commercial compound feeds. In contrast, the diets of cattle, sheep, goats and horses consist predominantly of forages, but their daily ration may be supplemented with other feed materials (cereal grains, cereal by-products and vegetable proteins) and supplements where the nutritional need of the animal cannot be met from forages alone (see Section 5), either as individual feeds, loose mixtures of feeds or manufactured compound feeds.

As discussed in Section 4, no data on the Ni content of compound feeds for specific livestock categories were available with which to estimate exposure to Ni. The CONTAM Panel therefore adopted two approaches for estimating exposure.

Method A: The mean LB and UB concentrations for the compound feeds (1 027.5 and 1 028 µg/kg dry matter (DM), respectively, n = 317) and forage meal (650.8 and 651.5 µg/kg DM, respectively, n = 524) have been used to estimate exposure to Ni using intakes given in tables A1–A3 above. Calculated exposures using this approach (Method A) are given in Table 6 of Section 6 of the main text.

Method B: In view of the uncertainty that may arise from the assumptions made in method A, the CONTAM Panel have also calculated exposure using the Ni content for individual feed materials using example rations. For ruminant diets, where forages represent a significant proportion of the total diet, mean LB and UB concentrations of 650.8 and 651.5 µg/kg, respectively, have been assumed for forages. In order to identify ‘worst-case’ exposures, it has been also assumed that hydrogenated vegetable oils are included in the non-forage component of the ration, at a rate of 5 % in the diet,²⁶ and that the hydrogenated oils contain the maximum permitted level of Ni (5 mg/kg).

B.1. Cattle, sheep and goats

Estimated example non-forage feed contents in the diets for cattle, sheep, goats and horses are given in Table B1, together with the calculated mean LB and UB levels of the sum of Ni in these diets.

Table B1: Estimated example diet compositions of non-forage feed for cattle, sheep and goats, and the calculated mean lower-bound and upper-bound levels (µg/kg) of nickel in these diets

Feeds	Dairy: high yielding	Beef: intensive cereal	Beef: fattening	Sheep: lactating	Goats: lactating	Goats: fattening	Horses
Wheat	15			14			
Barley	19	55	36	16	24	20	
Oats					33	37	40
Maize							
SBM	5			5	10	10	
RSM	20	5	20	10	10	10	
Sunflower meal		5		5			
Peas							
Beans	5			10			10
Maize gluten feed	9	10	11				
Lucerne meal							
Fishmeal							
Wheatfeed	8	5	10	13	10	10	30
Oatfeed							12
Sugar beet pulp	8	10	12	15		2	
Molasses	3	2	3	4	4	3	

²⁶ Although inclusion rates vary between diets, this is a typical upper limit in most formulations.

Feeds	Dairy: high yielding	Beef: intensive cereal	Beef: fattening	Sheep: lactating	Goats: lactating	Goats: fattening	Horses
Hydrolysed vegetable oils	5	5	5	5	5	5	5
Minerals, vitamins etc.	3	3	3	3	4	3	3
Lower-bound (µg/kg)	1 567	2 411	961	1 793	2 711	1 765	1 781
Upper-bound (µg/kg)	1 567	2 412	962	1 793	2 711	1 766	1 781

SBM: soya bean meal; RSM: rapeseed meal.

B.2. Pigs, poultry and rabbits

Pig and poultry diets consist predominantly of cereals (wheat or maize) and vegetable proteins. Pig diets may also include more fibrous feeds, particularly for older animals. The estimated example feed compositions in the diets for pigs and poultry are presented in Table B2 together with the calculated mean LB and UB concentrations of Ni in these diets.

Table B2: Estimated example diet composition (% inclusion) for pigs and poultry, and the calculated mean lower-bound and upper-bound levels of the sum of nickel in these diets

Feeds	Pig starter	Pig finisher	Lactating sow	Broilers: starter	Broilers: grower	Laying hens	Turkeys: grower	Ducks: grower
Wheat	48	48	50	33	36	30	30	40
Barley	14	18	10				34	15
Maize				30	36	32		
SBM	22	11	16	25	15	22	15	25
RSM	3	4						
Lucerne meal						4	9	5
Wheatfeed	2	7	12		1			7
Molasses	3	4	4	3	3	3	3	
Hydrolysed vegetable oils	5	5	5	5	5	5	5	5
Minerals, vitamins etc.	3	3	3	4	4	4	4	3
Lower-bound (µg/kg)	3 598	3 185	3 342	3 375	3 185	3 652	3 371	3 836
Upper-bound (µg/kg)	3 599	3 186	3 343	3 375	3 186	3 653	3 372	3 837

SBM: soya bean meal; RSM: rapeseed meal.

Although there are no published standard rations for rabbits, in a typical French commercial rabbit compound feed, the main ingredients were sunflower meal (20 %), dried lucerne (19.1 %), wheat/maize bran (18.3 %), barley (17.6 %), sugar beet pulp (11.9 %) and beans (10.4 %) (T. Gidenne, 2011, personal communication). Assuming the mean LB and UB concentrations above, estimated dietary LB and UB Ni concentrations are 3 073 µg/kg, respectively.

B.3. Fish

A wide range of diets is used for commercially farmed fish in Europe. A wide range of diets is used for commercially farmed fish in Europe. However, the salmon diet composition described in Table A6 has been used as being representative of commercial fish feed produced (Berntssen et al., 2010). Based on this formulation, the calculated mean LB and UB Ni concentrations for farmed fish are 5.0 and 9.0 µg/kg, respectively.

B.4. Companion animals (dogs and cats)

In typical French commercial pet foods, the cereals used are wheat, maize, barley, rice, maize gluten based on data compiled from six and seven different food brands for dogs and cats, respectively,

collected from pet food stores and veterinary clinics (J.M. Fremy, 2011, personal communication). The amounts of cereals in the premium quality and the standard quality dog food are 45 % and 65 %, respectively; in cat foods cereals and cereal by-products represented 40 % in premium quality food and 55 % in the standard quality food (B.M. Paragon, 2011, personal communication).²⁷

Assuming 65 % and 55 % cereal grains, their products and by-products in standard dog and cat foods, respectively, and the LB and UB concentrations given above, the mean LB and UB concentrations of Ni in cat and dog diets are given below in Table B4.

Table B4: The calculated mean lower bound (LB) and upper bound (UB) concentrations of the nickel in these diets

		Cats	Dogs
Mean concentration (µg/kg dry matter)	LB	2 650	2 650
	UB	2 651	2 651

²⁷ Based on statistics of 2010 of the French Association of Pet Food Manufacturers (FACCO), <http://www.facco.fr/>

Appendix C. Exposure of livestock and companion animals to nickel from water consumed

Mean LB and UB concentrations of Ni in 18 800 samples of drinking water (0.1 and 1.1 µg/L, respectively) have been reported (EFSA CONTAM Panel, 2015). Within species water consumption can vary considerably, influenced largely by ambient temperature but also to diet composition and level of activity and productivity. However, data for livestock have been published by a number of national authorities and summarised in OMAFRA (2007).

Table C1: Water intake and its contribution to overall nickel (Ni) exposure by livestock and companion animals

Animal species	Water intake, L/day	Ni from water, µg/day	Total Ni intake, µg/day	Ni from water as % of total exposure
Dairy: high yielding	133	146.3	32,461	0.45
Beef: intensive cereal	41	45.1	24,205	0.19
Beef: fattening	41	45.1	9,243	0.49
Sheep: lactating	32.5	35.8	5,027	0.71
Goats: lactating	10	11.0	9,283	0.12
Goats: fattening	10	11.0	2,670	0.41
Horses	7	7.7	16,293	0.05
Pig starter	2	2.2	3,603	0.06
Pig finisher	9	9.9	9,570	0.10
Lactating sow	20	22.0	20,072	0.11
Chickens for fattening	0.4	0.4	407	0.11
Laying hens	0.25	0.3	440	0.06
Turkeys for fattening	0.75	0.8	1,350	0.06
Ducks for fattening	1.1	1.2	540	0.22
Rabbits	0.64	0.7	459	0.15
Cats	0.15	0.2	159	0.10
Dogs	1.2	1.3	954	0.14

Appendix D. Overview of relevant nickel transfer studies

Table D1: Overview of relevant nickel (Ni) transfer studies

Animal species (age, weight; animal number)	Ni species	Ni exposure conditions	Ni concentration in animal product (wet weight)	Reference
Cattle (calves, 13–21 weeks; 3 animals)	NiCO ₃	0.4 g, 1.3 g, 1.6 g Ni/day for 8 weeks	No increase in liver and heart; Increase in kidney for 1.6 g Ni per day	O'Dell et al. (1971)
Cattle (calves, 50 days of age, initial weight 74 kg; 30 animals)	NiCl ₂	0, 5 mg Ni/kg diet 10 – 14.5 % protein For 140 days	10 % protein <u>Muscle:</u> control 54 mg/kg ± 5 µg/kg DW 5 mg/kg 65 ± 6 µg/kg DM <u>Liver:</u> control 38 ± 4 µg/kg DW 5 mg/kg 90 ± 18 µg/kg DM <u>Kidney:</u> control 48 ± 8 µg/kg DW 5 mg/kg 654 ± 120 µg/kg DM 12,25 % protein <u>Muscle:</u> control 54 ± 4 µg/kg DW 5 mg/kg 56 ± 5 µg/kg DM <u>Liver:</u> control 38 ± 4 µg/kg DW 5 mg/kg 56 ± 4 µg/kg DM <u>Kidney:</u> control 51 ± 4 µg/kg DW 5 mg/kg 306 ± 58 µg/kg DM 14,5 % protein <u>Muscle:</u> control 46 ± 5 µg/kg DW 5 mg/kg 49 ± 4 µg/kg DM <u>Liver:</u> control 46 ± 11 µg/kg DW 5 mg/kg 60 ± 11 µg/kg DM <u>Kidney:</u> control 46 ± 5 µg/kg DW 5 mg/kg 286 ± 83 µg/kg DM	Spears et al. (1984)
Cattle (8–12 month; 41 animals)	Not specified, soil and forage rich in Ni	Soil 5.91–940 mg Ni/kg DM forage 11.1–39.3 mg Ni/kg DM	<u>Muscle:</u> below LOD <u>Kidney:</u> ND –1758 mg/kg <u>Liver:</u> ND –33.5 mg/kg correlation Ni kidney, soil and forage	Miranda et al. (2009)
Cattle	Not specified	Farm near industrial plant	<u>Muscle:</u> 0.7 mg/kg <u>Liver:</u> 1.14 mg/kg	Skalicka et al. (2012)
Cattle and goat (no distinction reported)	Not specified	Village near polluted drain in Pakistan	<u>Meat:</u> 2.4–4.5 mg/kg <u>Kidney:</u> 0.8–2.4 mg/kg <u>Liver:</u> 3.2 mg/kg	Tabinda et al. (2013)
Cattle (cows)	Not specified	Livestock feed, conventional farm in Central Greece	<u>Muscle:</u> Ni below 0.02 mg/kg <u>Liver:</u> Ni below 0.02 mg/kg <u>Kidney:</u> Ni below 0.02 mg/kg	Makridis et al. (2012)

Table continued overleaf.

Table D1: Overview of relevant nickel (Ni) transfer studies (continued)

Animal species (age, weight; animal number)	Ni species	Ni exposure conditions	Ni concentration in animal product (wet weight)	Reference
Pigs (1 day old; 30 animals)	NiCl ₂	21 days low Ni (0.16 mg/kg) liquid milk based diet supplemented with 0, 5 or 25 mg Ni/kg DM	<u>Liver</u> : control 87 ± 2 µg/kg DM 5 mg/kg 127 ± 28 µg/kg DM 25 mg/kg 183 ± 14 µg/kg DM <u>Kidney</u> : control 151 ± 34 µg/kg DM 5 mg/kg 645 ± 157 µg/kg DM 25 mg/kg 1187 ± 85 µg/kg DM	Spears et al. (1984)
		21 days low Ni (0.16 mg/kg) liquid milk based diet and subsequently 28 days dry diet supplemented with 0, 5 or 25 mg Ni/kg DM	<u>Liver</u> : control 190 ± 52 µg/kg DM 5 mg/kg 245 ± 60 µg/kg DM 25 mg/kg 248 ± 12 µg/kg DM <u>Kidney</u> : control 138 ± 15 µg/kg DM 5 mg/kg 218 ± 20 µg/kg DM 25 mg/kg 808 ± 52 µg/kg DM <u>Muscle</u> : control 125 ± 17 µg/kg DM 5 mg/kg 118 ± 33 µg/kg DM 25 mg/kg 215 ± 23 µg/kg DM	
Pigs (initial weight 17.6 kg; 56 animals)	Not specified, sludge containing 538 mg Ni/kg, 115 mg Cd/kg, 4200 Zn mg/kg DM was supplied in liquid form to land to grow corn	Ni in control (C) corn: 0.46 mg/kg DM Ni in diet 1.6 mg kg/dry weight Ni in sludge amended corn: 2.40 mg/kg DM Ni in diet 3.3 mg/kg DM ; growth trial was terminated when pigs weighed 90 kg	<u>Muscle</u> : C corn 0.94 mg/kg DW SA corn 0.94 mg/kg DW <u>Liver</u> : C corn 0.97 mg/kg DW SA corn 0.94 mg/kg DW <u>Kidney</u> : C corn 2.12 mg/kg DW SA corn 4.02 mg/kg DW	Lisk et al. (1982)

Table continued overleaf.

Table D1: Overview of relevant nickel (Ni) transfer studies (continued)

Animal species (age, weight; animal number)	Ni species	Ni exposure conditions	Ni concentration in animal product (wet weight)	Reference
Pigs (initial weight 60 kg; 33 animals)	Not specified, sludge containing 32 mg Ni/kg DM	Pigs were fed corn- soybean grower diets containing 0, 10 or 20 % sewage sludge for 12 month control diet: 4 mg Ni/kg DM diet 10 %: 5 mg Ni/kg DM diet 20 %: 8 mg Ni/kg DM	Ni in milk and tissues of diets fed sows: <u>Milk</u> : control 0.1 mg/L 10 % or 20 % 0.1 mg/L <u>Muscle</u> : control 4 mg/kg DW 10 % 11 mg/kg DW 20 % 21 mg/kg DW <u>Liver</u> : control 3 mg/kg DW 10 % 14 mg/kg DW 20 % 23 mg/kg DW <u>Kidney</u> : control 5 mg/kg DW 10 % 10 mg/kg DW 20 % 20 mg/kg DW	Beaudouin et al. (1980)
Pigs (36 weanling pigs)			Ni in weanling pigs of 1 st litters: <u>Muscle</u> : control < 0.1 mg/kg DW 10 % < 0.1 mg/kg DW 20 % 20 mg/kg DW <u>Liver</u> : control < 0.01 mg/kg DW 10 % < 0.01 mg/kg DW 20 % < 0.01 mg/kg DW <u>Kidney</u> : control < 0.1 mg/kg DW 10 % < 0.1 mg/kg DW 20 % 24 mg/kg DW	
			Ni in weanling pigs of 2nd litters: <u>Muscle</u> : control 0.9 mg/kg DW 10 % 1.9 mg/kg DW 20 % 1.5 mg/kg DW <u>Liver</u> : control 0.9 mg/kg DW 10 % 2.9 mg/kg DW 20 % 1.7 mg/kg DW <u>Kidney</u> : control 0.7 mg/kg DW 10 % 1.9 mg/kg DW 20 % 2.1 mg/kg DW	

Table continued overleaf.

Table D1: Overview of relevant nickel (Ni) transfer studies (continued)

Animal species (age, weight; animal number)	Ni species	Ni exposure conditions	Ni concentration in animal product (wet weight)	Reference
Pigs (144 weanling pigs fed until a weight of 80– 90 kg)			Ni in growing-finishing swine of 1st litters: <u>Muscle</u> : control 2.3 mg/kg DW 10 % 2.5 mg/kg DW 20 % 3.2 mg/kg DW <u>Liver</u> : control 5.2 mg/kg DW 10 % 3.2 mg/kg DW 20 % 3.4 mg/kg DW <u>Kidney</u> : control 4.2 mg/kg DW 10 % 7.7 mg/kg DW 20 % 4.7 mg/kg DW	
			Ni in growing-finishing swine of 2nd litters: <u>Muscle</u> : control < 0.1 mg/kg DW 10 % 1.2 mg/kg DW 20 % 3.1 mg/kg DW <u>Liver</u> : control < 0.1 mg/kg DW 10 % 0.7 mg/kg DW 20 % 6.6 mg/kg DW <u>Kidney</u> : control < 0.1 mg/kg DW 10 % 0.9 mg/kg DW 20 % < 0.1 mg/kg DW	
Rabbits (adult; 15 animals)	NiCl ₂	Pellets supplemented with 0, 50 or 500 mg Ni/kg for 24 days	<u>Muscle</u> : 0.02 ± 0.01 mg/kg DM 0.07 ± 0.01 mg/kg DM 0.15 ± 0.02 mg/kg DM	Bersényi et al. (2004)
Adult lake whitefish (4 years of age; 72 animals)	NiSO ₄	Diets supplemented with 0, 10, 100 and 1000 µg Ni/kg wet weight for 10, 31 or 104 days Diet 1: 1.1 ± 0.02 mg Ni/kg wet weight. Diet 2: 12 ± 0.12 mg Ni/kg wet weight Diet 3: 110 ± 0.68 mg Ni/kg wet weight Diet 4: 1100 ± 3.0 mg Ni/kg wet weight	<u>Muscle</u> : D1 (10 days): 0.04 ± 0.00 mg/kg D4 (10 days): 0.08 ± 0.00 mg/kg D4 (31 days): 0.14 ± 0.01 mg/kg D4 (104 days): 0.20 ± 0.01 mg/kg	Ptashynski et al. (2001)

b.w.: body weight; C: Control; DM: dry matter; DW: dry weight; LOD: limit of detection; ND: not detectable.

Appendix E. Contribution of animal derived food to nickel exposure

Table E1: Distribution of the average contribution from food of animal origin to human dietary exposure to nickel across different age classes and dietary surveys. Estimates of mean and 95th percentile dietary exposure derived only from consumption of food of animal origin are also described.

Age class	Dietary surveys ^(c)	Average contribution (%)	Mean dietary exposure (µg/kg b.w. day)	95th dietary exposure (µg/kg b.w. day)
		(LB–UB) ^(a)	(LB–UB) ^(a)	(LB–UB) ^(a)
Infants	NUTRICHILD	13.1–25.7	0.4–1.4	1.3–5.4
	INRAN_SCAI_2005_06	40.5–40.5	1.7–2.6	– ^(b)
	Regional_Flanders	29.1–31.8	2.8–3.8	– ^(b)
Toddlers	NUTRICHILD	15.1–23.1	1.1–2.4	2.0–4.5
	DIPP_2003_2006	19.7–26.3	1.4–2.4	3.1–5.5
	DONALD_2006_2008	16.6–19.7	0.9–1.4	1.6–2.5
	INRAN_SCAI_2005_06	18.7–22.8	1.2–1.9	– ^(b)
	VCP_kids	17.6–23.5	1.4–2.4	3.1–4.8
	enKid	13.0–19.7	1.4–2.6	– ^(b)
	Regional_Flanders	22.0–26.4	1.6–2.4	3.7–4.9
Other children	NUTRICHILD	15.4–20.2	1.2–2.0	2.2–3.5
	SISP04	10.3–13.9	0.8–1.3	1.5–2.3
	Danish_Dietary_Survey	18.1–21.4	1.0–1.6	1.7–2.7
	DIPP_2003_2006	23.8–29.1	1.5–2.5	2.5–4.3
	STRIP	10.8–15.1	0.7–1.2	1.2–2.0
	INCA2	11.7–15.2	1.0–1.5	1.6–2.4
	DONALD_2006_2008	12.4–16.1	0.7–1.2	1.5–2.3
	Regional_Crete	11.7–16.3	0.9–1.4	1.6–2.5
	INRAN_SCAI_2005_06	15.6–18.2	0.9–1.3	2.0–2.6
	EFSA_TEST	9.4–13.0	0.5–0.8	1.0–1.7
	VCP_kids	14.5–20.8	1.0–1.8	2.1–3.6
	NUT_INK05	13.4–19.6	1.0–1.7	1.7–2.9
	enKid	12.6–18.2	1.0–1.7	2.0–3.3
	NFA	15.2–19.7	0.9–1.5	1.6–2.6
	Diet_National_2004	10.0–12.1	0.3–0.5	0.7–1.0
Adolescents	Childhealth	9.9–12.1	0.3–0.4	0.7–0.9
	SISP04	12.2–15.8	0.6–0.9	1.1–1.7
	Danish_Dietary_Survey	18.5–21.0	0.6–0.9	1.1–1.6
	INCA2	11.2–14.1	0.4–0.7	0.8–1.3
	National_Nutrition_Survey_II	10.6–12.6	0.3–0.4	0.6–0.9
	INRAN_SCAI_2005_06	15.7–17.8	0.5–0.7	1.2–1.5
	EFSA_TEST	10.0–13.8	0.3–0.6	0.8–1.2
	AESAN_FIAB	15.4–20.0	0.5–0.8	1.1–1.4
	NUT_INK05	12.0–16.7	0.6–0.9	1.1–1.6
	enKid	12.3–16.7	0.6–0.9	1.1–1.7
	NFA	14.6–18.4	0.5–0.9	1.0–1.5
Adults	Diet_National_2004	11.3–13.6	0.3–0.5	0.7–1.0
	SISP04	15.5–18.9	0.4–0.6	0.8–1.1
	Danish_Dietary_Survey	13.9–16.5	0.4–0.6	0.7–1.0

Age class	Dietary surveys ^(c)	Average contribution (%)	Mean dietary exposure (µg/kg b.w. day)	95th dietary exposure (µg/kg b.w. day)
		(LB–UB) ^(a)	(LB–UB) ^(a)	(LB–UB) ^(a)
	FINDIET_2007	15.6–20.6	0.4–0.6	0.7–1.2
	INCA2	13.5–16.4	0.4–0.6	0.7–1.0
	National_Nutrition_Survey_II	10.9–12.9	0.3–0.4	0.6–0.9
	National_Repr_Surv	16.3–19.7	0.5–0.7	1.0–1.3
	NSIFCS	14.6–16.5	0.3–0.5	0.6–0.8
	INRAN_SCAI_2005_06	14.9–16.9	0.4–0.5	0.7–1.0
	EFSA_TEST	12.5–16.3	0.3–0.4	0.6–1.0
	DNFCS_2003	12.1–16.3	0.4–0.6	0.7–1.2
	AESAN	15.9–20.0	0.5–0.7	1.2–1.5
	AESAN_FIAB	17.0–21.2	0.5–0.8	0.9–1.3
	Riksmaten_1997_98	10.1–14.3	0.3–0.5	0.5–0.9
	NDNS	11.6–14.0	0.3–0.4	0.5–0.7
	Diet_National_2004	11.6–13.6	0.3–0.4	0.6–0.9
	Danish_Dietary_Survey	14.4–17.0	0.4–0.6	0.6–1.0
Elderly	FINDIET_2007	15.2–20.6	0.3–0.5	0.6–1.0
	INCA2	14.2–16.4	0.4–0.5	0.6–0.9
	National_Nutrition_Survey_II	12.0–14.0	0.3–0.4	0.5–0.8
	National_Repr_Surv	15.9–19.0	0.4–0.6	0.8–1.0
	INRAN_SCAI_2005_06	14.3–15.8	0.3–0.4	0.6–0.8
	Diet_National_2004	11.5–13.4	0.3–0.4	0.6–0.8
Very elderly	Danish_Dietary_Survey	15.0–18.3	0.4–0.6	– (b)
	INCA2	13.1–15.6	0.3–0.5	0.5–0.8
	National_Nutrition_Survey_II	11.5–13.5	0.3–0.4	0.5–0.8
	National_Repr_Surv	15.1–17.8	0.4–0.6	0.7–0.9
	INRAN_SCAI_2005_06	12.5–14.3	0.3–0.4	0.5–0.6

b.w.: body weight; LB: Lower bound, UB: Upper bound.

(a): Information on the dietary surveys is given in the Guidance of EFSA ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (EFSA, 2011b).

(b): The 95th percentile for samples with less than 60 observations is not shown as the results may not be statistically robust (EFSA, 2011b).

ABBREVIATIONS

AAS	Atomic absorption spectrometry
AFRC	Agricultural and Food Research Council
APAG	European Oleochemicals and Allied Products Group
ARC	Agricultural Research Council
ATSDR	Agency for Toxic Substances and Disease Registry
BMDL ₁₀	Lower 95 % confidence limit for a benchmark response at 10 % extra risk
b.w.	Body weight
CAS	Chemical Abstracts Service
CONTAM Panel	EFSA Panel on Contaminants in the Food Chain
DM	Dry matter
DW	Dry weight
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EU RAR	European Union Risk Assessment Report
FAAS	Flame atomic absorption spectrometry
FCE	Feed conversion efficiency
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
FEFAC	European Feed Manufacturers Federation
FEDIOL	European Vegetable Oil and Protein Meal Industry
FoodEx	EFSA Food classification and description system for exposure assessment
FSA	UK Food Standards Agency
FYM	farm-yard manure
GFAAS	Graphite furnace atomic absorption spectrometry
IARC	International Agency for Research on Cancer
ICP-MS	Inductive coupled plasma mass spectrometry
ICP-OES	Inductive coupled plasma optical emission spectrometry
IOM	Institute of Medicine
IPCS	International Programme on Chemical Safety
IOS	International Organisation for Standardization
LB	Lower bound
LC	Left-censored
LD ₅₀	Lethal dose 50 %
LOD	Limit of detection
LOQ	Limit of quantification
LT	Lake trout
LWF	Lake whitefish
MLs	Maximum levels
MOE	Margin of exposure
MRL	Maximum risk level
Ni	Nickel
Ni ⁰	Metallic nickel
NiCl ₂	Nickel chloride
NiCO ₃	Nickel carbonate
[Ni(H ₂ O) ₆] ²⁺	hexaquonickel ion
NiSO ₄	Nickel sulphate
NOAEL	No observed adverse effect level
NRC	National Research Council of the National Academies (US)
OECD	Organisation for Economic Co-operation and Development
RBC	Red blood cells
RfD	Reference Dose
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment), the Netherlands

ROS	Reactive oxygen species
RSM	Rapeseed meal
SBM	Soya bean meal
SCD	Systemic contact dermatitis
TDI	Tolerable daily intake
TERA	Toxicology Excellence for Risk Assessment
UB	Upper bound
UK	United Kingdom
US	United States Environmental Protection Agency
WHO	World Health Organization
WHO/IPCS	World Health Organization/International Programme on Chemical Safety